UNIL | Université de Lausanne
Faculté de biologie et de médecine

DEPARTEMENT DE BIOCHIMIE
RAPPORT 2006-2007

Département de Biochimie, Faculté de Biologie et de Médecine,
Université de Lausanne, Ch. des Boveresses 155, CH-1066 Epalinges,
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Couverture: Detection of a new malaria antigen (green) in Plasmodium infected red blood cells. Parasite nuclei in blue. (Courtesy of G. Corradin)

EN HOMMAGE AU PROFESSEUR HENRI ISLIKER

1922 - 2007

Le 1er août 2007, le Département de Biochimie a eu la tristesse de perdre son fondateur et premier directeur, le Prof. Henri Isliker.

Son oeuvre scientifique très riche a été centrée sur l'étude de la structure et des propriétés des immunoglobulines et des composés du système du complément, à une époque où apparaissaient les premières méthodes d'identification, de séparation et d'analyse des protéines. Mais nous lui devons avant tout la création d'un centre de recherche unique en Suisse, regroupant diverses institutions, qui en commun ont largement contribué à la renommée dont jouit l'Université de Lausanne dans le domaine des sciences de la vie.

Né en 1922 à Genève, Henri Isliker étudie la chimie à l'Université de Berne et après une thèse de doctorat en biochimie, il passe cinq années au sein du "Department of Biophysical Chemistry " de la Harvard Medical School. Il retourne ensuite à Berne où il est nommé Privat Docent avant d'être appelé par la Faculté de Médecine de l'Université de Lausanne pour créer et diriger l'Institut de Biochimie dès 1958.

Durant les dix ans qui suivent, et tout en assumant la responsabilité de l'enseignement de la biochimie au sein de la Faculté de Médecine de notre Université, il déploie une activité intense permettant de mettre en place le premier institut universitaire de Suisse spécialisé en immunochimie et immunologie fondamentale. Grâce à son enthousiasme, sa lucidité, sa vision moderne de la recherche scientifique et sa force de conviction, il fonde et anime simultanément l'Institut Suisse de Recherches Expérimentales sur le Cancer (ISREC), puis dans la foulée permet la création d'une unité lausannoise de l'Institut Ludwig de Recherche sur le Cancer, dont la double orientation vers l'immunologie et l'oncologie reflète ses intérêts scientifiques. Dans le même esprit d'ouverture, et grâce aux liens qu'il a su établir avec de grandes institutions internationales, il installe également dès 1963 le Centre OMS de Référence sur les Immunoglobulines, qui deviendra par la suite le WHO Immunology Research and Training Center. En 30 années d'activité, cette unité a non seulement contribué à la formation de plus de 850 boursiers médecins et biologistes des pays en développement, mais est aussi à l'origine de la mise en place au sein du Département de Biochimie de programmes de recherche sur certaines maladies parasitaires ou transmissibles et sur le développement de vaccins. Conscient du rôle crucial de cette unité, Henri Isliker a lui-même activement participé à de nombreux enseignements souvent dispensés dans les pays concernés.

Avec une énergie hors du commun, cet esprit ambitieux et fédérateur a été capable de rassembler des scientifiques d'horizons et de caractères parfois très différents et complémentaires, tout en leur laissant la liberté totale de conduire leurs projets.

La personnalité chaleureuse de notre ami Henri, sa grande modestie et sa volonté de toujours placer en priorité la qualité de la recherche et le soutien aux chercheurs, en particulier aux jeunes de la relève, furent pour nous ses élèves, ses collègues et ses amis une exemple rayonnant. Que cet esprit qui l'a animé dans toutes ces réalisations inspire celles et ceux qui ont la responsabilité de poursuivre l'oeuvre exceptionnelle de ce pionnier.

Claude Bron
Professeur Honoraire

Le Département de Biochimie (DB) de l'Université de Lausanne est entré dans sa cinquantième année d'existence, ce qui est non seulement l'occasion d'évaluer le chemin parcouru, mais aussi de se projeter dans l'avenir.

Au cours de ses 50 années d'activité, le DB s'est focalisé sur des thématiques importantes couvrant les domaines de l'immunologie, du cancer et des maladies infectieuses. Localisé tout d'abord au voisinage du CHUV, puis sur le site du Centre biomédical d'Epalinges, le DB a focalisé son activité de recherche sur des thématiques importantes couvrant les domaines de l'immunologie, du cancer et des maladies infectieuses. Au cours de ces cinquante années, le DB s'est acquis dans ces domaines une renommée internationale de par la qualité de ses travaux scientifiques. Ce statut est dû au dynamisme de ses collaborateurs, ainsi qu'aux liens étroits tissés avec d'autres institutions académiques présentes sur le site d'Epalinges (ISREC: Institut Suisse de Recherche Expérimentale sur le Cancer; LICR: Ludwig Institute for Cancer Research), créant ainsi des synergies bénéfiques à tous. De même, le DB a pu bénéficier d'une plate-forme technologique moderne et performante, résultant de la mise en commun des infrastructures de recherche de ces différentes institutions.

Mais si le DB est arrivé à ce niveau d'excellence, c'est avant tout grâce à l'esprit insufflé par son fondateur, le Professeur H. Isliker, qui malheureusement nous a quittés l'année passée. Nous tenons à rendre hommage à cette personnalité marquante, fondateur du DB, de l'ISREC et initiateur de la venue du LICR et d'un centre de formation et de recherche OMS à Lausanne. Il a su par son esprit visionnaire, ses contacts et sa détermination de tous instants placer notre Département dans une situation idéale pour son développement. Cet esprit a été repris par son successeur, le Prof. Claude Bron qui a su développer le Département et l'organiser autour de thématiques définies. Notre tâche a consisté, ces dernières années, à consolider l'acquis, à intégrer de nouveaux groupes de recherche, à gérer la croissance en donnant aux utilisateurs les meilleures conditions de travail possibles pour qu'ils puissent accomplir leur mission de recherche et d'enseignement et ainsi augmenter la performance et la visibilité du DB.

Le DB compte aujourd'hui plus de 120 collaborateurs (101 EPT) de 19 nationalités différentes. Douze groupes de recherche partagent nombre d'infrastructures et comblent leur budget par des financements externes conséquents. Plus de la moitié du budget du DB provient de fonds externes, tels le FNS, les programmes nationaux prioritaires, les projets de la communauté européenne, la commission pour la technologie et l'innovation. Ainsi, le DB participe activement à des projets nationaux et européens sans pour autant négliger ses relations avec les domaines cliniques dans des projets translationnels et avec l'industrie dans des projets de transfert de technologie. Le bon fonctionnement du Département et le bon état d'esprit qui y règne sont aussi visibles par le nombre de collaborations entre les groupes du Département, soulignant une volonté constante de travailler ensemble dès que l'occasion se présente.

Au cours de ces deux dernières années, peu de changements sont intervenus au sein du corps professoral du DB et les charges d'enseignement sont essentiellement les mêmes, bien qu'elles aient été adaptées au processus de Bologne.

Au niveau des nominations, nous soulignons celle de Pascal Schneider au titre de Maître d'enseignement et de recherche en septembre 2006 et la reconnaissance faite au centre OMS et au travail de Fabienne Tacchini-Cottier qui a été nommée Professeure associée *ad personam* en septembre 2007.

Des progrès importants en recherche ont été accomplis dans: l'utilisation d'un modèle, obtenu par transgénèse, de cellules dendritiques CD8+ tumorales conservant toutes les caractéristiques de cellules dendritiques, permettant ainsi une approche de génomique fontionnelle pour comprendre les mécanismes conduisant à l'histiocytose (Hans Acha-Orbea); la caractérisation des protéines de l'anémie de Fanconi impliquées dans les mécanismes de maintien de l'intégrité du génome lors de la réplication (Angelos Constantinou); une nouvelle approche basée sur une analyse bioinformatique du génome en combinaison avec des synthèses de peptides pour une identification systématique et la validation de candidats vaccins notamment dans le cas de la malaria (Giampietro Corradin); l'importance du gène Notch1 comme cible de p53 et comme gène suppresseur de tumeurs (Gian-Paolo Dotto); la caractérisation de la première enzyme protéolytique participant au programme de mort cellulaire du parasite *Leishmania* et la définition de facteurs de virulence dans les formes mucocutanées de leishmaniose (Nicolas Fasel); le rôle important des lymphocytes B comme présentateurs d'antigènes dans le développement d'une réponse de type Th2 lors d'une infection par le parasite *Leishmania major* (Pascal Launois); la fonction des cellules stromales dans les tissus lymphoïdes secondaires et leur importance dans l'homéostase et l'attraction des lymphocytes T (Sanjiv Luther); le développement de protéines bifonctionnelles CMH Class I ou apparentées, fusionnées à un fragment d'anticorps antitumeur, permettant de rediriger vers la tumeur la réponse immunitaire innée et adaptative.(Jean-Pierre Mach); l'identification et la cristallisation de la première polymerase polyphosphate, une enzyme essentielle pour les organismes unicellulaires et impliquée dans les interactions symbiotiques (Andreas Mayer); le traitement de la dysplasie ectodermique par utilisation d'un ligand de la famille TNF (Pascal Schneider); la démonstration d'un rôle distinct des neutrophiles suite à une infection par *Leishmania major* dans des souris susceptibles ou résistantes à l'infection ce qui influence directement la réponse immunitaire (Fabienne Tacchini-Cottier); les mécanismes d'activation et de survie des lymphocytes, et plus particulièrement de la protéine MALT1, un régulateur important qui pourrait devenir une cible thérapeutique pour le traitement de certains lymphomes (Margot Thome Miazza); la démonstration que l'inflammosome, qui est responsable de la production de l'interleukine-1, est impliqué dans la goutte, permettant une nouvelle approche thérapeutique ainsi que la caractérisation moléculaire du maintien du virus de l'hépatite C dans des cellules du foie (Jürg Tschopp).

Comme les années précédentes, plusieurs de ces travaux ont été publiés dans des périodiques à fort impact démontrant, une fois encore, que le Département a su maintenir son niveau d'excellence.

Les progrès réalisés dans les différents domaines d'activité ont aussi été reconnus par l'obtention de prix. En 2006, le Dr Christoph Reese (groupe A. Mayer) a reçu le prix d'Excellence du jeune chercheur (Commission des prix de la Faculté de Biologie et de Médecine (FBM)) et le Dr Etienne Meylan (groupe J. Tschopp) le prix de la Ville de Lausanne. Le Professeur Andreas Mayer a reçu le prix Friederich-Miescher en 2006 et le prix de la

Fondation Leenaards en 2007. Le Professeur Jürg Tschopp a reçu l'European Cell Death Award en 2006. En octobre de cette année, il recevra le prix de la Fondation Louis-Jeantet pour son travail sur la caractérisation des mécanismes impliqués dans l'inflammation. Cette distinction prestigieuse sera pour la première fois attribuée à un chercheur lausannois.

Les 19 thèses de doctorat soutenues durant ces deux dernières années, le nombre de publications parues souvent dans des journaux à fort impact, l'importance des apports financiers extra-budgétaires pour conduire les programmes de recherche, les distinctions reçues, le rôle déterminant joué par le Département dans la vie de la FBM et de l'UNIL sont autant de signes de sa vitalité. Certes, ceci résulte des compétences réunies de tous les collaborateurs, des supports techniques et administratifs mais surtout du privilège de pouvoir conduire les missions de recherche au sein d'un consortium d'instituts ayant des intérêts scientifiques convergents.

A la lecture du rapport d'activité, on pourrait donc croire que ces deux dernières années se sont passées sans heurts ni tracas et que notre Département a maintenu sa productivité et continué son développement sans se soucier du futur et de la relève.

Cette vision des choses n'est cependant pas complètement correcte. En effet, le départ de l'ISREC avait placé le DB dans une situation peu propice à son développement, remettant en question l'utilisation du site d'Epalinges et de ses infrastructures. Le Département s'était exprimé pour son maintien dans ses locaux d'Epalinges et avait milité pour l'arrivée de nouveaux groupes de recherche pour occuper les surfaces libérées par le départ de l'ISREC, permettant ainsi la création d'une masse critique scientifique suffisante dans ses domaines d'activité. Le récent rachat des bâtiments de l'ISREC par l'Etat de Vaud, la volonté et le soutien de la Direction de l'Université et du CHUV de réorganiser les sciences de la vie sur la place lausannoise en développant un centre d'immunologie à Epalinges ouvrent des perspectives prometteuses pour notre Département mais aussi pour l'ensemble de la recherche et la formation de la Faculté et du CHUV.

Dans ce consortium, le DB espère jouer un rôle prépondérant dans ses domaines de compétence en sachant que la venue de groupes performants du CHUV va renforcer l'identité du centre biomédical d'Epalinges et permettre une utilisation optimale des infrastructures à disposition.

Nicolas Fasel
Directeur

Personnel rétribué par l'UNIL: 52.15 EPT (équivalent plein temps)
* Personnel rétribué par des fonds privés: 48.5 EPT

1. DEPARTEMENT DE BIOCHIMIE (décembre 2007)

- **Corps professoral**

H. Acha-Orbea	Professeur associé
C. Bron	Professeur honoraire
A. Constantinou *	Professeur assistant (boursier FNS)
G. Corradin	Professeur associé
G.P. Dotto	Professeur
N. Fasel	Professeur associé
S. Luther *	Professeur assistant (boursier FNS)
J.P. Mach	Professeur honoraire
J. Mauël	Professeur honoraire
A. Mayer	Professeur
P. Schneider	Maître d'enseignement et de recherche
M. Thome *	Professeur assistant (boursier FNS)
J. Tschopp	Professeur

- Corps intermédiaire

K. Lefort	Maître-assistant
S. Masina	Maître-assistant
L. Otten	Maître-assistant

- Assistants

Postdoctorant(e)s

M. Bras	A. Donda *	C. Dostert *
M. Eckert *	L. Franzini Brunner *	F. Grosjean
S. Grossi *	G. Guarda	B. Hu
T. Kimura	A. Kündig	E. Logette *
S. Luke-Glaser *	M.C. Michallet *	S. Papin *
V. Petrilli *	M. Pieren	A. Posevitz *
J. Romero *	R. Torgler	R. Zhou

Doctorant(e)s

G. Agak	T. Baars	C. Bossen
M. Britschgi *	W. Chemnitz *	S. Chevrier
A. D'Osualdo *	R. Dawaliby	M. Delannoy
S. Fuertes Marraco	K. Gari	I. Gonzalez
S. Hailfinger	L. Heinz *	A. Ives
C. Lambertini *	A. Link *	A. Mayor
P. Menu *	L. Michaillat	R. Moser *
F. Rebeaud *	M. Rebsamen	G. Restivo
D. Rossi *	S. Siegert	E. Silayuv
F. Staehli *	K. Stirnemann	B. Strasser
M. Tapernoux	T. Vogt *	H. Zalila
M. Zieger *		

Stagiaires

S. Balam	S. Olugbile *	G. Mascré *
M. Rengifo	G. Ruzzante	E. Suzarte *

- **Personnel technique**

Laborantin(e)s

G. Badic	V. Calpini *	E. Castillo
R. Castillo Arias *	V. Comte *	C. Décaillet *
C. Desponds	S. Favre *	K. Fournier *
M. Guzzardi *	A. Hautier	S. Hertig
C. Lavanchy	G. Frank *	C. Mattmann
J. Perrin *	M. Perroud	F. Prevel *
M. Reinhardt	M. Rosa *	A. Schmidt Luther *
A. Tardivel *	J. Vazquez *	L. Willen *

Aide laborantine

L. Morgado *

Apprenties laborantines

L. Almeida	T. Cardoso	L. Mury

- **Services généraux**

Administration

S. Aslan (75%)	F. Flejszman (75%)	M. Jayet Herzstein

Atelier

D. Roy

Laverie

M. Ethenoz (90%)	S. Peter (10%)

Magasin central et Travaux pratiques

M. Margot * (80%)	P. Margot	U. Margot (50%)

2. CENTRE OMS DE RECHERCHE ET DE FORMATION EN IMMUNOLOGIE

P. Launois *	Directeur et professeur *ad personam*
F. Tacchini-Cottier *	Co-directrice des cours OMS et professeur associé *ad personam*
C. Ronet *	Postdoctorante
C. Allenbach *	Postdoctorante
F. Auderset *	Doctorante
M. Charmoy	Doctorante
Y. Hauyon La Torre *	Laborantine
F. Flejszman *	Secrétaire

3. PROTEIN AND PEPTIDE CHEMISTRY FACILITY

C. Servis *	Cheffe de projet
L. Rodrigues *	Laborantin
V. Studer *	Stagiaire

4. Départs

- **Corps intermédiaire**

E. Meylan	(03.2007)	C. Peters	(11.2007)

- **Assistants**

Postdoctorant(e)s

B. Delgado	(07.2007)	H. Everett	(03.2006)
I. Kolfschoten	(10.2007)	S. Lippens	(10.2006)
J. Martinez	(02.2007)	R. Narasimamurthy	(05.2007)
S. Pantano	(11.2006)	D. Rueda	(08.2006)
Q.G. Steiner	(06.2006)	V. Villard	(10.2007)
R. van Bruggen	(09.2006)		

Doctorant(e)s

L. Agostini	(08.2006)	M. Breton	(11.2007)
B. Brissoni	(01.2006)	P. Brunetto	(06.2006)
V. Cesson	(07.2006)	A. Corsinotti	(01.2007)
S. Cuenin	(08.2007)	S. Delacrétaz	(02.2007)
C. Genton	(09.2006)	D. Meier	(02.2006)
H. Neumann	(05.2006)	V. Swoboda	(03.2007)
A. Uttenweiler	(09.2006)	J. Wagner	(02.2006)

Stagiaires

L. Lozano	(09.2007)	B. Blasco	(02.2007)
C. Perez	(04.2006)	K. Monnat	(06.2006)
P. Önal	(09.2006)	M. Montecchio	(11.2007)
E. Roggli	(05.2007)		

- **Personnel technique**

Laborantin(e)s

K. Ingold	(01.2007)	C. Pavlin	(08.2007)
F. Penea	retraite (06.2006)	M. Rousseaux	retraite (11.2006)
E. Säuberli	(01.2006)	S. Streuli	(03.2006)

Apprenti(e)s laborantin(e)s

O. Froelicher	(08.2006)	A. Da Costa	(08.2006)

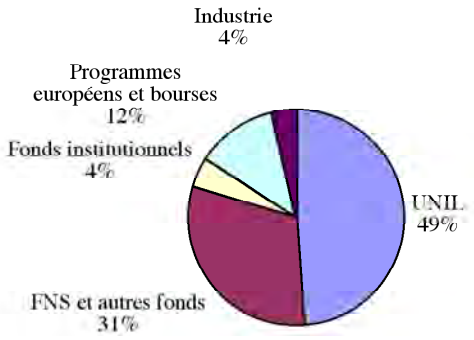
Stagiaires

S. Favre	(08.2007)	N. Jacquier	(09.2007)
E. Maeder	(07.2006)	E. Rauccio	(06.2007)
J. Tapparel	(07.2007)	J. Widmer	(08.2007)

- **Services généraux**

V. Debellemanière	(02.2006)	L. Skupienski	(06.2007)

RESSOURCES 2007



Ressources externes: CHF 6'690'916
Etat de Vaud/UNIL: CHF 6'309'421

ENSEIGNEMENTS

	Nombre d'heures/an				Etudiants	Nombre étudiants
	C	S	APP	TP		
1ère année						
Biochimie	34	2	-	-	médecine/	420
					biologie	139
Génétique	10	-	-	-	médecine/	420
					biologie	139
2ème année						
Cellule	12	14	-	53	médecine	196
Immunité	-	-	16	-	médecine	
Immunologie	8	-	-	-	biologie	80
Métabolisme	21	-	-	-	médecine/	196
					biologie	80
Métabolisme	21	-	-	-	biologie	80
3ème année						
Bachelor	56	6	-	32	biologie	42
4ème année						
Master	90	10	-	50	biologie	6
	Travail d'initiation (4 mois); travail de Master (4 mois)					
5ème année						
Master	Travail de Master (4 mois)					

C = cours; S = séminaires; APP = apprentissage par problème;
TP = travaux pratiques

Enseignement postgradué sous l'égide de l'OMS, organisé en collaboration et avec le soutien de la Direction suisse du Développement et de la Coopération internationale (DDC)

- Immunologie, Vaccinologie et Biotechnologie appliquées aux maladies infectieuses. 18 participants.
  - Cours de spécialisation en maladies infectieuses. Sénégal, 17 et 18.03.2006
  - Cours avancé. Epalinges, 19.05 - 22.06.2007
  - Cours avancé. Epalinges, 25.06 - 07.07.2007
- Immunology, Vaccinology and Biotechnology applied to infectious diseases. 18 participants.
  - Advanced course. Epalinges, 06.09 - 21.10.2006
  - Advanced course. Bangladesh, 29.11 - 12.12.2006
  - Advanced course. Epalinges, 04.09 - 18.10.2007

THESES DE DOCTORAT

Anja Apel: The lysosome as target for therapeutic intervention.

George Agak: Immunological and protective properties of novel malaria blood stage peptide antigens.

Laetitia Agostini Bigger: Characterization of NALP2 and NALP3 IL-1b processing inflammasomes.

Cindy Allenbach: Les neutrophiles et le TNF: deux composants importants de la réponse immunitaire induite par l'infection avec *Leishmania Major*.

Valérie Cesson: Development and evaluation of antibody-MHC/peptide conjugates as a new form of cancer immunotherapy.

Solange Cuenin: Gestion du stress: Les stratégies de la cellule face aux dommages à l'ADN et le rôle des isoformes de la protéine PIDD.

Kerstin Gari: Fanconi anemia and genome stability: The role of FANCM.

Céline Genton Activation des cellules B de manière T-dépendante chez les souris $LatY^{136F}$: une réponse polyclonale responsable d'une autoimmunité systémique.

Iveth Jimena Gonzalez Jimenez: La métacaspase de *Leishmania major* et la mort cellulaire programmée.

Greta Guarda: Generation and function of mouse central memory and effector memory T cells.

Alexander Link: Lymphoid stroma in health and disease.

Dominik Meier: Role of cytokines in secondary lymphoid organ development.

Etienne Meylan: Mécanismes moléculaires impliqués dans l'activation de NF-KB et la défense antivirale.

Heinz Neumann: Structure and function of the VTC complex of S. cerevisiae.

Mélanie Revaz-Breton: Le rôle des récepteurs Toll-like 9 dans le développement d'une réponse immunitaire Th1/Th2 suite à l'infection par *Leishmania major* dans un modèle murin.

Elizabeth Bongfen Silayuv: Characterization of the immunological properties of *Plasmodium falciparum* and *Plasmodium berghei* circumsporozoite proteins: antibody responses and recognition by specific $CD8^+$ T cells.

Ralph Torgler: Role of hepatocyte wounding on *Plasmodium* liver-stage development and survival.

Andreas Uttenweiler: Identification of novel factors of micro-autophagocytosis in yeast.

Julian Wagner: Nouveaux modèles génétiques et cellules mésenchymales comme outil pour améliorer l'immunité anti-tumorale.

Hans Acha-Orbea,
Associate Professor

Interest: Role of dendritic cells and B cells during immune responses.
Ph.D. in 1984 with Hans Hengartner and Rolf Zinkernagel in Zurich followed by a postdoctoral fellowship (1985-1989) in Hugh McDevitt's lab in Stanford, USA. Research topics: Roles of T cell receptor and MHC in autoimmunity.
Start fellow, assistant and later associate member at the Ludwig Institute for Cancer Research (1989-2003). Research themes: retroviral superantigens, virus-host interaction.
Joined the Department of Biochemistry in 1994. Research themes: virus-host interaction, dendritic cells and differentiation of B cells during immune responses.

Group members 2006-2007

Christine Lavenchy, Technician
Muriel Rosa, Technician
Luc A. Otten, Maître Assistant
Dirk Claeys, Postdoctoral fellow
Frederic Grosjean, Postdoctoral fellow
Xavier Martinez, Postdoctoral fellow
Quynh-Giao Steiner, Postdoctoral fellow
Stéphane Chevrier, PhD student
Silvia Fuertes, PhD student
Céline Genton, PhD Student
Julian Wagner, PhD Student
Simone Crausaz, Master Student
Maude Greutert, Master Student

Transgenic mice developing dendritic cell tumors, a tool for investigating dendritic cell immunology and a model for human histiocytosis

Characterization of a new histiocytosis model; oncogenic tranformation of dendritic cells in transgenic mice

Dendritic cells (DC) are the key regulators of immune response and immune tolerance. In the absence of microbes and danger signals they induce immune tolerance towards presented peptides whereas after microbial infection they direct the immune response towards the infecting microbe. They can imprint the site of infection as well as the effector function required for elimination of the microbe on the responding T cells. Different subset of DC exist in mice with partly understood functions. Amongst the conventional DC the two major subsets are $CD8^+$ and $CD11b^+$ DC respectively. The former are more efficient at CD8 T cell priming and crosspresentation, the latter for CD4 T cell priming. Plasmacytoid DC are responsible for large amounts of type I interferon production immediately after pathogen encounter and later for antigen presentation. In addition several subsets of tissue DC such as Langerhans cells of the skin exist.

Achievements

We have obtained a first model for human histiocytosis by overexpressing the SV40 large T oncogene specifically in DC using the CD11c promoter (Steiner et al. Blood 2008). Depending on the expression levels of the transgene in transgenic founders mice show DC transformation at 4 or 12 months respectively with full penetrance (Figure 1 A). The forming tumors are all $CD8^+$ DC and we were able to show that always mature splenic nonactivated $CD8^+$ DC transform after about 3 months or 6-12 months in the two transgenic founder lines. The reason for this preferential transformation of $CD8^+$ DC might lie in the higher steady state proliferation of this subset in normal mice. Despite the fact that Langerhans are thought to be at the origin of human Langerhans cell

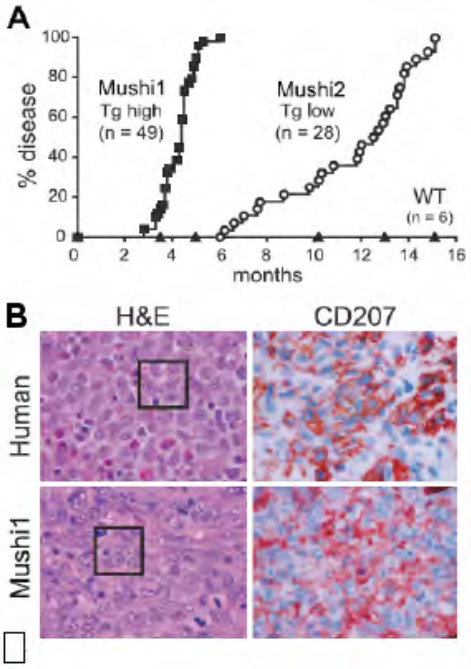


Figure 1:
A) *Survival kinetics of the high (Mushi1) and low (Mushi 2) transgene expressing large T transgenic mice*
B) *Comparision of human and mouse disease in H&E stains (left) and anti-Langerin immunohistology (right).*
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TRANSGENIC MICE DEVELOPING DENDRITIC CELL TUMORS, A TOOL FOR INVESTIGATING DENDRITIC CELL IMMUNOLOGY AND A MODEL FOR HUMAN HISTIOCYTOSIS

Selected publications

- Steiner, Q.G., Otten, L.A., Hicks, M.J., Kaya, G., Sauberli, E., Lavenchy, C., Grosjean, F., Beermann, F., MacClain, K.L. and Acha-Orbea, H. (2008) In vivo Transformation of Mouse Conventional CD8a+ Dendritic Cells Leads to Progressive Multisystem Histiocytosis. Blood 111, 2073-2082.
- Wang, Y., Kissenpfennig, A., Mingueneau, M., Richelme, S., Perrin, P., Chevrier, S., Genton, C., Lucas, B., DiSanto, J.P., Acha-Orbea, H., Malissen, B. and Malissen, M. (2008) Th2 lymphoproliferative disorder of LatY136F mutant mice unfolds independently of TCR-MHC engagement and Is insensitive to the action of Foxp3 regulatory T cells. J. Immunol. 180, 1565–1575.
- Link, A., Vogt, T.K., Favre, S., Britschgi, M.R., Acha-Orbea, H., Hinz, B., Cyster, J.G. and Luther, S.A. (2007) Fibroblastic reticular cells in lymph nodes regulate the homeostasis of naive T cells. Nat. Immunol. 8, 1255-1265.
- Meier, D., Bornmann, C., Chappaz, S., Schmutz, S., Otten, L.A., Ceredig, R., Acha-Orbea, H. and Finke, D. (2007) Ectopic lymphoid-organ development occurs through interleukin 7-mediated enhanced survival of lymphoid-tissue-inducer cells. Immunity 26, 643-654.
- Acha-Orbea, H., Shakhov, A.N. and Finke, D. (2007) Superantigens in health and disease. immune response to MMTV infection. Frontiers in Bioscience Edition "Immune response to MMTV infections". 2, 1594-1609.
- Genton, C., Wang, Y., Izui, S., Malissen, B., Delsol, G., Fournié, G.J., Malissen, M. and Acha-Orbea, H. (2006) The T_H2 lymphoproliferation developing in Lat^{Y136F} mutant mice triggers polyclonal B-cell activation and systemic autoimmunity. J. Immunol. 177, 2285-2293.
- Otten, L.A., Lavanchy, C., Barras, E., LeibundGut-Landmann, S., Kos-Braun, I., Huarte, J., Steimle, V., Acha-Orbea*, H. and Reith*, W. (2006) Revisiting in vivo the specificity of the MHC class II transactivator, CIITA. * Equal contribution. Eur. J. Immunol. 36, 1548-1558.

histiocytosis the non-Langerhans cell-derived $CD8^+$ tumors in our mice show striking similarities to human disease. Tumors express high levels of Langerin as in human disease, a marker thought to be specific for Langerhans cells until recently (Figure 1B). These results raise the possibility that in a subset of human disease transformation/accumulation of conventional splenic DC might be involved. The tumors maintain most of the features of freshly isolated $CD8^+$ DC such as cytokine production after Toll-like receptor stimulation, antigen (cross)-presentation and expression of specific transcription factors and surface markers (Figure 2). Stable cell lines can be easily derived from the tumors that maintain all the features of non-activated DC. These tools represent the first stable cell lines with the features of normal DC and are valuable tools for designing experiments to understand DC function.

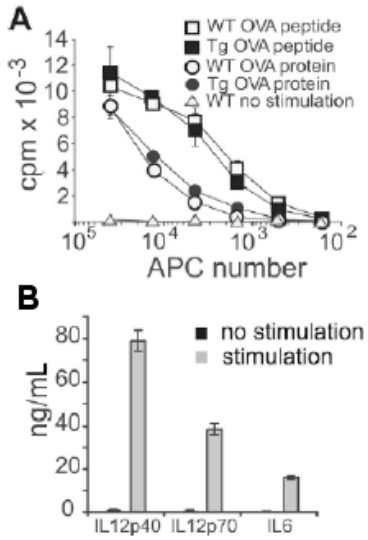

Figure 2:
A) *Comparable antigen (cross)-presentation of tumor cells and wild type DC OTI anti-Ova T cell receptor transgenic T cells were stimulated with Ova protein or Ova peptide and dDC and proliferation was measured. WT: wild type, Tg: Tumoral DC from transgenic mice*
B) *Cytokine production after Toll-like receptor stimulation.*
This research was originally published in Blood: Steiner et al. 2008:111:2073-82.
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Current projects

To better understand DC transformation we have undertaken Affimetrix gene arrays in normal DC and during the transformation process. These results are being analyzed now and will give targets potentially involved in transformation (Figure 3). Using lentiviral transduction yielding in overexpression or siRNA mediated inhibition of expression the role of selected molecules will be tested in the cell lines. Selected genes will be tested for expression in human Langerhans cell histiocytosis samples to validate the model and find new targets for therapy.
We have recently made the observation that the cell lines die by apoptosis after Toll-like receptor stimulation. We will test the behavior of wild type DC to see whether they follow the same rules. We also will investigate the induction of pro-and anti-apoptotic molecules in cell lines and wild type DC to understand the molecular mechanisms leading to apoptosis in DC. Lentiviral transduction and use of transgenic and knockout mice will address the roles of the found molecules. In addition we will generate models transforming Langerhans cells, $CD11b^+$ DC or plasmacytoid DC.

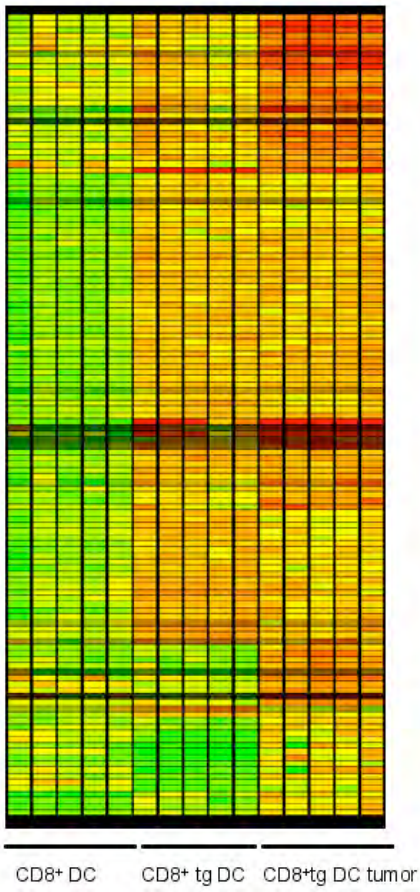


***Figure 3**: Affimetrix DNA array analysis of 5 samples per group.: Significant differences in gene expression due to transgene expression and transfromation. tg: large T transgenic*

Plasma cell differentiation during an immune response

During a T cell-mediated immune response, antigen-specific B cells become activated via DC-primed antigen-specific T cells to form germinal centers and develop into memory and effector cells (plasma cells). Plasma cells migrate back to the bone marrow where they compete for niches where their long-term survival is induced. In the absence of bone marrow homing, plasma cells are short-lived.

Achievements and current projects

We have recently described a new plasma cell marker, CD93. This C-type lectin is expressed during the early stages of B cell development in the bone marrow and the spleen. We have shown that it is upregulated during plasma cell development. Its expression is maintained on long-lived plasma cells in the bone marrow niche. Little is know about the function of this molecule, nothing about its role in the plasma cell survival/homing. We have recently obtained the CD93 KO mice and test them now for the early and late plasma cell response *in vivo* and in vitro.

Angelos Constantinou
Assistant Professor

Interest: DNA repair and genome instability in relation to tumorigenesis.
PhD in 1998. Professor Stuart Clarkson, University Medical Centre, Geneva. Research topic: nucleotide excision repair, structure function analysis of the XPG repair protein.
Postdoctoral studies (1999-2002) at Cancer Research UK, London, in the laboratory of Professor Steve C. West. Research topic: the branch migration and resolution of recombination intermediates in mammalian cells.
Joined the Department of Biochemistry in 2003.

Group members 2006-2007

Chantal Décaillet, Technician
Simona Grossi, Postdoctoral fellow
Sarah Luke-Glaser, Postdoctoral fellow
Mathieu Delannoy, PhD student
Kerstin Gari, PhD student

Mechanisms of tumour suppression by the Fanconi anemia pathway

Background

In recent years, a causal relationship has been established between replication stress, genome instability and tumour formation. We aim to understand how genetic information is faithfully transmitted from one cell generation to the next. Our current focus is the molecular functions of proteins involved in Fanconi anemia.

Fanconi anemia

Fanconi anemia (FA) is a genetically heterogeneous recessive disorder characterised by congenital abnormalities, bone marrow failure and cancer predisposition. Mutations in any of 13 genes can lead to Fanconi anemia. Accumulating evidence has revealed that Fanconi anemia proteins function in a DNA damage network during the S phase of the cell cycle. FA cells are exquisitely sensitive to interstrand DNA crosslinking agents, and exhibit high incidence of both spontaneous and damage induced chromosomal aberrations. We are using a combination of biochemical a cell biological approaches to elucidate the roles of FA proteins in DNA replication, DNA damage signalling and DNA repair.

FANCM is a branchpoint translocase

We discovered that FANCM binds with high affinity and specificity to branched DNA molecules, such as model replication forks and Holliday junctions, and promotes the migration of their junction point in an ATPase dependent manner. Our working hypothesis is that FANCM remodels stalled replication forks, thereby allowing repair enzymes to gain access to the replication-blocking lesion.

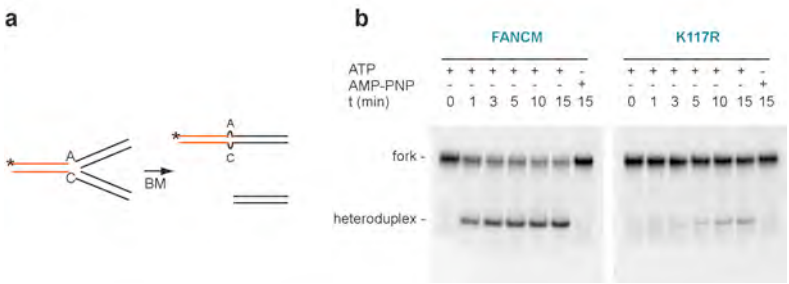


***Figure 1**: (a) Schematic representation of a model replication fork, and the heteroduplex products arising from branchpoint translocation. Asterisks indicate ^{32}P-label at the DNA 5'-end. (b) Autoradiographs showing branchpoint translocation. Reactions with FANCM (2 nM) were performed for the indicated periods at 37°C in the presence of ATP or AMP-PNP, as indicated. To verify that branchpoint translocation was catalysed by FANCM, similar reactions were conducted with an ATPase deficient K117R FANCM protein (2 nM).*

Impact of the FA pathway on the dynamics of DNA replication

To study the role of Fanconi anemia proteins in DNA replication, we are using a DNA combing procedure: cellular replicons are pulse-labelled with distinct halogenated nucleotides (recognised by specific antibodies) and DNA molecules are isolated, stretched out and aligned on a glass surface. This powerful approach allows the study of the many parameters that determine the duplication of our genome, such as the rate of progression of replication forks, the stalling or the restart of stalled forks, and the firing of new origins of replication at any given time.

Selected publications

- Gari, K., Décaillet, C., Stasiak, A.Z., Stasiak, A. and Constantinou, A. (2008) The Fanconi Anemia Protein FANCM Can Promote Branch Migration of Holliday Junctions and Replication Forks. Mol. Cell, 29, 141-148.
- Constantinou, A., Chen, X.B., McGowan, C.H. and West, S.C. (2002) Holliday junction resolution in human cells: two junction endonucleases with distinct substrate specificities. EMBO J. 21, 5577-5585.
- Constantinou, A., Davies, A.A. and West, S.C. (2001) Branch migration and Holliday junction resolution catalyzed by activities from mammalian cells. Cell 104, 259-268.
- Constantinou, A., Tarsounas, M., Karow, J.K., Brosh, R.M., Bohr, V.A., Hickson, I. D. and West, S.C. (2000) Werner's syndrome protein (WRN) migrates Holliday junctions and co-localizes with RPA upon replication arrest. EMBO Rep. 1, 80-84.
- Constantinou, A., Gunz, D., Evans, E., Lalle, P., Bates, P.A., Wood, R.D. and Clarkson, S.G. (1999) Conserved residues of human XPG protein important for nuclease activity and function in nucleotide excision repair. J. Biol. Chem. 274, 5637-5648.

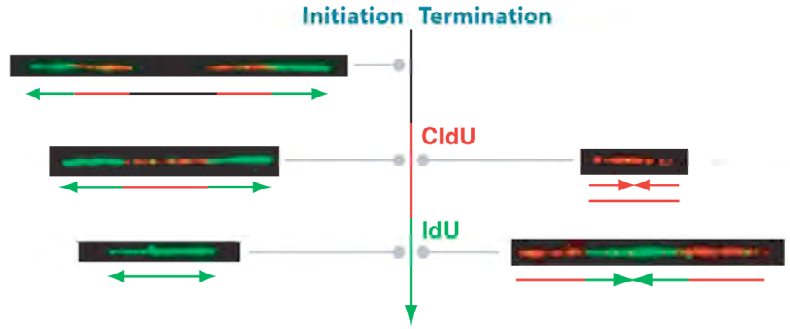


***Figure 2**: Replication patterns from asynchronous cells after sequentially labelling with CldU (red) and IdU (green). Five representative replication patterns are shown. The patterns depend on when an origin fires (initiation) or replication forks merge (termination) relative to the labelling period, considering that replication is bidirectional.*

A very stimulating aspect of research on genome stability is that it gives us insight into fundamental molecular processes that can be targeted for the treatment of tumours. Tumour cells experience constitutive replication stress and heavily rely on functional DNA damage tolerance pathways for survival. This research may contribute to the design of rational chemotherapy and radiotherapy protocols.

Giampietro Corradin,
Associate Professor

Giampietro Corradin graduated in chemistry at the University of Padua and received his PhD degree in chemistry from the University of California, Santa Barbara, after completion of a thesis on the structure and function of cytochrome c. After a post-doctoral position in biochemistry at Dartmouth Medical School, Hanover, New Hampshire, he continued his training in molecular immunology at the National Jewish Hospital, Denver, Colorado. He joined the Institute of Biochemistry in 1979 where he is Associate Professor.

Jean-Pierre Mach,
Professor Emeritus

Jean-Pierre Mach received his medical degree at the University of Geneva and trained from 1963 to 1967 as a postdoctoral fellow at the Massachussetts General Hospital in Boston. He specialized in the field of tumor markers at the Department of Biochemistry of our University where he became professor in 1976. From 1978 to 1986, he was associate director of the Lausanne branch from the Ludwig Institute. At his retirement from a double appointment at ISREC and our University, he joined Prof. G. Corradin's group to pursue his research with Dr Alena Donda, project leader, on antibody-mediated targeting of antigenic MHC class I complexes and MHC related molecules on tumor cells.

MALARIA: Search of new antigens for the development of protective vaccines

Malaria is a world-wide parasitic disease which affects millions of people especially young children and pregnant women. Among the measures directed toward the prevention of this disease, vaccines represent a cost-effective approach. Identification of molecules important in the elicitation of a protective immune response and their use in experimental animals to be followed by testing in human volunteers have been a constant goal of our laboratory. Malaria is a parasitic disease transmitted during the blood meal of infected mosquitoes which inoculate sporozoites into the mammalian host. Within minutes, sporozoites invade hepatocytes and develop into merozoites intracellularly by asexual schizogony. The merozoites then invade red blood cells, producing the various symptoms associated with the disease. The life-cycle is completed when gametocytes are ingested during the blood meal of the mosquito vectors. Upon repeated infections, partial antibody-dependent immunity directed against the erythrocytic stage is elicited in humans.

Protective immunity against malaria can be obtained by immunizing mice and humans with irradiation-attenuated sporozoites. This immunity is the result of the effect of neutralizing antibodies recognizing free sporozoites in the blood stream and of CD4+ and CD8+ T cells which prevent the development of the parasite hepatic forms. Experiments performed in B cell deficient mice have demonstrated that, despite the absence of anti-sporozoite antibodies, protection is induced by irradiated sporozoite immunization. This suggests that T cells specific for proteins present in the intracellular hepatic stage play a predominant role in protection. Therefore, one of the aim in malaria vaccine research is to mimic the protective immune response induced by injection of irradiated sporozoites. On the other hand the role and interplay of hepatocytes and parasites, respectively, in eliciting a T cell response is not very well known. To this effect we studied the following topics:

Antigen processing and presentation of sporozoite antigens by infected hepatocytes

We are investigating how primary hepatocytes from BALB/c mice process the CSP of P. berghei after live sporozoite infection, and present CSP-derived peptides to specific H-2Kd-restricted $CD8^+$ T cells in vitro. We show that both infected and trasversed primary hepatocytes process and present the CSP of P. berghei. The processing pathway was found to involve the proteasome and antigen transport through a post-Endoplasmic Reticulum compartment and aspartic proteases. These results suggest that sporozoite antigens are processed and presented in vitro and possibly in vivo via the classical MHC class I pathway. In the presence of specific $CD8^+$ T cells, sporozoite-infected hepatocytes were resistant to CTL-induced apoptosis, even though they present sporozoite antigens. Thus, the developing parasite is mainly eliminated not by apoptosis of infected hepatocytes, but by secretion of IFN-γ/TNFα upon recognition of infected hepatocytes by specific T cells.

Activation of hepatocytes by live sporozoites

Plasmodium sporozoites traverse several host cells before infecting hepatocytes. In the process, the plasma membranes of the cells are ruptured resulting in the release of cytosolic factors into the microenvironment. This released endogenous material is highly stimulatory/immunogenic and can serve as a danger signal initiating

Group members 2006-2007

Géraldine Frank, Technician
Marga Rousseaux, Technician
Leonor Morgado, Assistant technician
Teresa Cardoso, Apprentice
Jackeline Romero, Researcher
Viviane Villard, Postdoctoral fellow
George Agak, PhD student
Silayuv Bongfen, PhD student
Francisco Estevez, PhD student
Ralph Torgler, PhD student
Saïdou Balam, Trainee
Sope Olugbile, Trainee
Edith Suzarte, Trainee
Joël Tapparel, Trainee

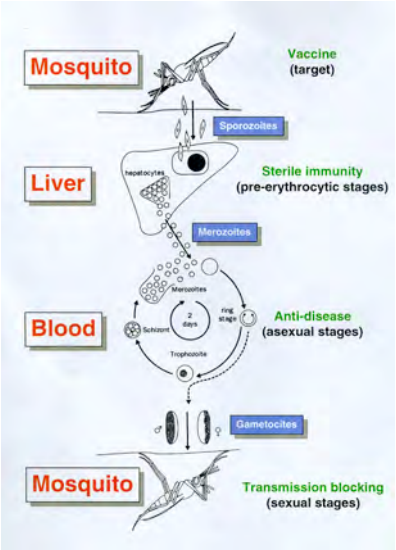
Selected publications

- Torgler, R., Bongfen, S.E., Romero, J.C., Tardivel, A., Thome, M. and Corradin, G. (2008) Sporozoite-mediated hepatocyte wounding limits Plasmodium parasite development via MyD88-mediated NF-kappa B activation and inducible NO synthase expression. J. Immunol. 180(6), 3990-9.
- Agak, G.W., Bejon, P., Fegan, G., Gicheru, N., Villard, V., Kajava, A.V., Marsh, K. and Corradin, G. (2008) Longitudinal analyses of immune responses to Plasmodium falciparum derived peptides corresponding to novel blood stage antigens in coastal Kenya. Vaccine 26(16), 1963-71.
- Villard, V., Agak, G.W., Frank, G., Jafarshad, A., Servis, C., Nébié, I., Sirima S.B., Felger, I., Arevalo-Herrera, M., Herrera, S., Heitz, F., Bäcker, V., Druilhe, P., Kajava, A.V. and Corradin, G. (2007) Rapid identification of malaria vaccine candidates based on alpha-helical coiled coil protein motif. PLoS ONE 2(7), e645.
- Corradin, G., Villard, V. and Kajava, A.V. (2007) Protein structure based strategies for antigen discovery and vaccine development against malaria and other pathogens. Endocr. Metab. Immune Disord. Drug Targets. 7(4), 259-65.
- Bongfen, S.E., Torgler, R., Romero, J.F., Renia, L. and Corradin, G. (2007) Plasmodium berghei-infected primary hepatocytes process and present the circumsporozoite protein to specific CD8+ T cells in vitro. J. Immunol. 178(11), 7054-63.
- Corradin, G. (2007) Peptide based malaria vaccine development: personal considerations. Microbes Infect. 9(6), 767-71.

distinct responses in various cells. Thus, we aimed to evaluate the effect of leakage of cell material during Plasmodium infection on responses of cultured mouse primary hepatocytes. We observed that wounded cell-derived cytosolic factors activate NF-κB - a main regulator of host inflammatory responses - in cells bordering wounded cells, which are potential host cells for final parasite infection. This activation of NF-κB occurred shortly after infection and led to a reduction of infection load in a time-dependent manner in vitro and in vivo, an effect that could be reverted by addition of the specific NF-κB inhibitor BAY11-7082 or by using Spect-/- sporozoite mutants. We provide further evidence that NF-κB activation induces inducible nitric oxide synthase (iNOS) expression in hepatocytes, and this is, in turn, responsible for a decrease in Plasmodium-infected hepatocytes. Furthermore, primary hepatocytes from MyD88$^{-/-}$ mice showed no NF-κB activation and iNOS expression upon infection, suggesting a role of the Toll/IL-1 receptor family members in sensing cytosolic factors. Indeed, lack of MyD88 significantly increased infection in vitro and in vivo. Thus, host cell wounding due to sporozoite migration induces inflammation with a detrimental effect for the parasite.

Erythrocytic vaccine discovery and development

New malaria vaccine candidates were identified based on α-helical coiled coil protein motif predicted to be present in the parasite erythrocytic stage. The corresponding synthetic peptides are expected to mimic structurally "native" epitopes. Ninetyfive peptides 30-40 amino acids long were chemically synthesized. All selected antigens were then tested in ELISA assay using a panel of sera from donors living in different malaria endemic areas. Recognition of these synthetic antigens varied from few to 100 %, thus identifying a number of new potential vaccine candidates.
Affinity purified antibodies recognize the infected red blood cells and peptides are, generally, immunogenic in mice. Further characterization of these proteins using mouse specific or affinity purified human antibodies was performed. In order to select the most appropriate protective antigens, a series of assays like antibody dependent cytotoxic inhibition (ADCI) and growth inhibition assay (GIA) were employed to determine the biological properties of specific antibodies. Results obtained to date demonstrate that our bioinformatics/chemical synthesis approach can lead to the identification of new proteins that can be targets of potential vaccines and/or drugs in a relatively short time frame. In addition, the antigen selection mechanism is, in principle, of general application, and can be applied to any other pathogen where protection is mediated by antibodies.



Malaria cycle

Group members 2006-2007

Karine Fournier, Technician
Alena Donda, Project leader
Valérie Cesson, PhD student
Kathrin Stirnemann, PhD student
Benjamin Blasco, Trainee
Shirley Favre, Trainee

CANCER: Antibody-mediated tumor targeting of MHC class I and MHC-related molecules

Taking advantage of our experience in tumor targeting with antibodies and in T lymphocyte cytotoxicity, we designed a new type of conjugates, consisting of antibody fragments coupled to Major Histocompatibility Complexes (MHC) containing antigenic viral peptides. We demonstrated that Fab fragments from different anti-tumor marker antibodies, conjugated to MHC/viral peptides, can target these antigens on the surface of cancer cells and induce their efficient lysis by virus specific cytotoxic T lymphocytes. We developed several in vivo syngeneic tumor models in viral infected mice and demonstrated the feasibility of this new immunotherapy strategy. In view of these promising results, we are presently testing in parallel similar approaches applied to non-polymorphic MHC related molecules, such as CD1d or MICA, to attract at the tumor site effector cells from the innate immune system such as NKT and NK cells.

Antibody-MHC/viral peptide conjugates for cancer cells targeting

Antibody-based cancer immunotherapy exploits the cell surface expression by cancer cells of tumour-associated antigens (TAA). The generation and engineering of high affinity anti-TAA monoclonal antibodies (mAbs) as native proteins, or as carriers for targeting radioactivity, toxins or cytokines to tumor cells have made important progresses and antibody-based cancer therapy is now effective for lymphoma, ovarian and breast cancer (for review, Mach 2002). Despite these very encouraging clinical results, however, one should remain aware that the injection of anti-tumor mAbs, such as anti-HER2 (herceptin) or anti-CD20 (Rituximab), when used as a single modality therapy, usually leads to partial tumor regression and mAb treatment needs to be combined with chemotherapy.

A second important strategy is to exploit the cellular immune response to cancer cells and T cell based cancer immunotherapy essentially focuses on the development of cancer vaccines to optimize the potent cytotoxicity of T lymphocytes against tumor derived antigens. Although a number of clinical trials are being conducted, this approach is still facing problems of tumor escape, like absence of costimulatory molecules or downregulation of MHC Class I expression on tumor cells.

Recently, we, and others, have developed an alternative strategy whereby anti-TAA mAbs are used to target on tumor cells recombinant MHC class I/viral peptide complexes in order to induce the killing of target cells by viral specific CD8 T lymphocytes. This strategy exploits the specific tumor localization of high affinity anti-TAA antibodies, which allows the coating of the cancer cells with class I molecules filled with a highly antigenic peptide. Altogether, this attractive therapeutic approach has clear advantages over the vaccination with poorly antigenic autologous tumor antigens. First, it will not be affected by the loss of endogenous MHC Class I expression by the tumour. Second, viral antigens are generally more potent than tumor antigens to generate cytotoxic T cells. Third, the lack of accessory molecules on the cancer cells, often reported as the cause of tolerance/anergy of T cells specific for TAA, will not affect the present strategy since the preexisting anti-viral memory T cell pool can be fully activated by a challenge with the appropriate vaccine at the time of treatment.

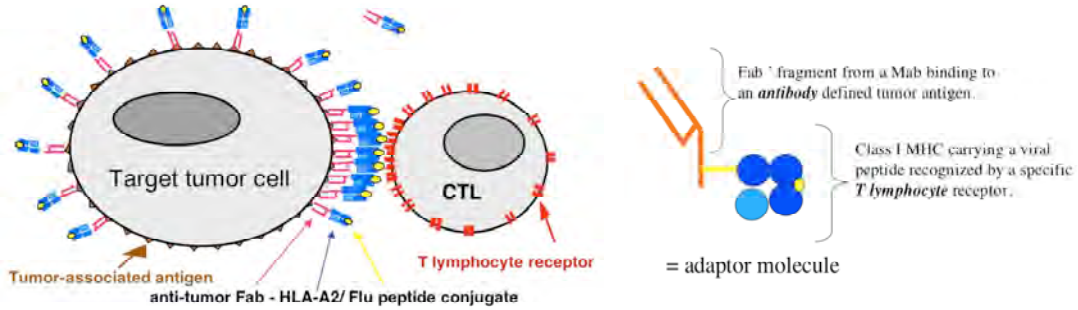
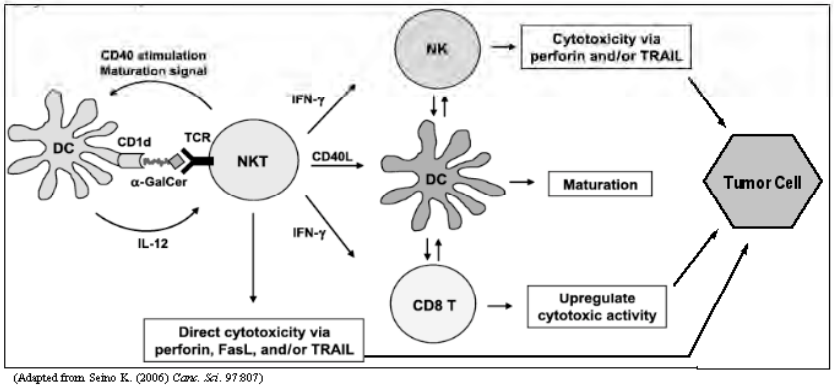


***Figure 1**: Left part: schematic description of the binding to TAA and oligomerization of antibody Fab' fragment-MHC/peptide conjugates on the surface of a tumor cell, which induce the cooperative binding of several T-cells receptors leading to activation of the T effector lymphocyte, which will kill the target tumor cell. Note that the free conjugates do not bind to T cells due to the low affinity of monomeric MHC/peptide complexes to individual T cells receptors. Right part: enlarged schematic structure of the antibody Fab' fragment-class I MHC/peptide conjugate which represents an "adaptor molecule" able to transform an antibody-defined antigen into an antigen recognized by T lymphocytes.*

We have demonstrated for the first time in an entirely in vivo models of syngeneic carcinoma that this new tumor immunotherapy strategy can function in immunocompetent mice, using two different viral models, the Lymphochoriomeningitis virus (LCMV) and the Influenza virus (Flu), in mice grafted with subcutaneous tumor or lung metastasis, respectively. We have improved the quality of the conjugates by UV crosslinking the antigenic peptide into the groove of the MHC molecule, in collaboration with I. Luescher from the Ludwig Institute, so that the stability of the complex is greatly increased in vivo. We showed that systemic injection of such conjugates can efficiently induce tumor cell killing and tumor growth inhibition by the specific CD8+ CTLs generated by the viral infection (Cesson et al., 2006).

Antibody-CD1d fusion bifunctional molecule for targeting innate immunity to cancer cells

The aim of the present project is to activate at the tumor site effector cells of the innate immune system, such as CD1d-restricted NKT cells, by an antitumor antibody-mediated delivery of the CD1d non-polymorphic MHC class I related molecule loaded with the ligand α-galactosylceramide (α-GalCer) or analogs. Practically, this strategy is based on the development of a bifunctional molecule consisting of the CD1d-α-GalCer complex as the activating part, genetically fused to the tumor targeting part, such as single chain antibody fragments with high



(Adapted from Seino K. (2006) *Canc. Sci.* 97:807)

***Figure 2**: **NKT cells as transactivators of the innate and adaptive immune response.** The glycolipid ligand α-GalCer presented by CD1d expressed on DC will activate NKT cells. In turn, activated NKT cells will rapidly secrete IFNγ and upregulate CD40L. These events will lead to NK activation, DC maturation and subsequent development of antigen-specific T lymphocytes, possibly against an existing tumor.*

Selected publications

- Stirnemann, K., Romero, J.F., Robert, B., Cesson, V., Besra, G.S., Zauderer, M., Wurm, F., Corradin, G., Mach, J.P., MacDonald, H.R. and Donda, A. (2008) Sustained activation and tumor targeting of NKT cells using a CD1d-anti-HER2 scFv fusion protein induce antitumor effects in mice. J. Clin. Invest. 118, 994-1005.
- Larbouret, C., Robert, B., Navarro-Teulon, I., Thèzenas, S., Ladjemi, M.Z., Morisseau, S., Campigna, E., Bibeau, F., Mach, J.P., Pèlegrin, A. and Azria, D. (2007) In vivo therapeutic synergism of anti-epidermal growth factor receptor and anti-HER2 monoclonal antibodies against pancreatic carcinomas. Clin. Cancer Res. 13, 3356-3362.
- Heer, A.K., Shamshiev, A., Donda, A., Uematsu, S., Akira, S., Kopf, M. and Marsland, B.J. (2007) TLR signaling fine-tunes anti-influenza B cell responses without regulating effector T cell responses. J. Immunol. 178, 2182-2191.
- Cesson, V., Stirnemann, K., Robert, B., Luescher, I., Filleron, T., Corradin, G., Mach, J.P. and Donda, A. (2006) Active antiviral T-lymphocyte response can be redirected against tumor cells by antitumor antibody x MHC/viral peptide conjugates. Clin. Cancer Res. 12, 7422-7430.
- Buchegger, F., Antonescu, C., Delaloye, A.B., Helg, C., Kovacsovics, T., Kosinski, M., Mach, J.P. and Ketterer, N. (2006) Long-term complete responses after 131I-tositumomab therapy for relapsed or refractory indolent non-Hodgkin's lymphoma. Br. J. Cancer 94, 1770-1776.
- Fattah, O.M., Cloutier, S.M., Kundig, C., Felber, L.M., Gygi, C.M., Jichlinski, P., Leisinger, H.J.,Gauthier, E.R., Mach, J.P. and Deperthes, D. (2006) Peptabody-EGF: a novel apoptosis inducer targeting ErbB1 receptor overexpressing cancer cells. Int. J. Cancer 119, 2455-2463.

affinity for a tumor associated antigen (TAA). This antitumor antibody-CD1d molecule will recruit and activate at the tumor site CD1d-restricted NKT cells known for their cytoxicity and potent ability to activate NK cells,as shown in the schematic diagram of figure 2.

This strategy is similar to the one described above for tumor targeting of classical MHC class I-antigen and induction of specific cytotoxic T lymphocytes (CTL). However, replacing MHC Class I molecule with the MHC related CD1d has additional advantages. First, the CD1d protein is a monomorphic antigen presenting molecule, which would allow, in future clinical applications, the development of a single conjugate to treat a majority of patients in contrast to the polymorphic MHC Class I molecules. Second, even though the natural ligands of CD1d and the physiological role of NKT cells is still a matter of debate, an important antimetastatic activity of CD1d-NKT cells has been demonstrated in vitro and in vivo in the presence of the glycosphingolipid α-GalCer. Third, the fast activation of CD1d-NKT cells rapidly leads to the activation and proliferation of NK cells, which altogether greatly enhance cytotoxicity. Importantly, the tumor targeting part of the antibody-CD1d bifunctional molecule will restrict the recruitment of these cytotoxic effector cells at the tumor site and hence will increase efficacy, while limiting the known toxicity of an untargeted NKT and NK cell activation.

We have developed a genetic fusion of mouse β2 microglobulin - CD1d - anti-HER2 scFv, which is well produced by human embryonic kidney cells HEK293 in a transient transfection system. The fusion is well refolded as shown by binding to tumor cells expressing the HER2 antigen and by recognition with anti-CD1d antibody. The CD1d fusion is functional since it is able to activate NKT hybridoma, as demonstrated by the release of IL-2.

Our first original in vivo observation was that when αGalCer was loaded on the recombinant soluble CD1d molecule (αGalCer/sCD1d), repeated injections led to a sustained iNKT and NK cell activation associated with interferon γ secretion as well as with DC maturation. In contrast, it is known that a single injection of the free form of αGalCer leads to a short-lived iNKT cell activation followed by a long-term anergy, limiting its therapeutic use.

Most importantly, when the αGalCer/sCD1d fused to the anti-HER2 scFv antibody fragment was injected, potent inhibition of experimental lung metastasis (Fig.3) and established subcutaneous tumors was observed even when systemic treatment was started 2 to 7 days after the injection of HER2-expressing B16 melanoma cells, whereas at this time free αGalCer has no effect. Furthermore, we demonstrated that the anti-tumor activity of the CD1d-anti-HER2 fusion protein is associated with HER2-specific tumor localization and accumulation of iNKT, NK and T cells at the tumor site (Stirnemann et al. 2008).

Our results strongly suggest that targeting iNKT cells to the tumor site can activate a combined innate and adaptive immune response that may prove to be effective in cancer immunotherapy.

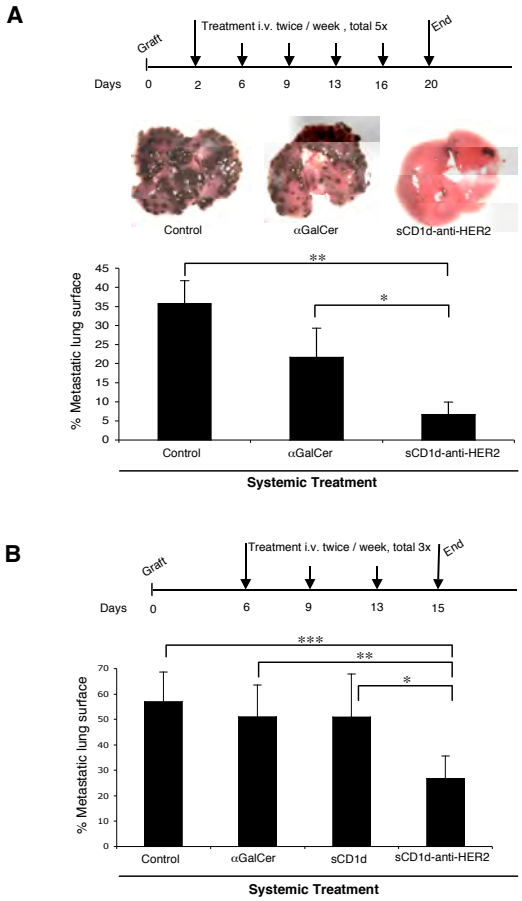


***Figure 3**: **In vivo anti-tumor activity - Systemic Treatment.** (A) Mice were grafted i.v. with 700'000 B16-HER2 cells and i.v. treatment was started 48 hours later. Mice were injected five times i.v. every 3 to 4 days (arrows) with either PBS (control), or equimolar amounts of αGalCer (0.4 μg), or αGalCer-loaded sCD1d-anti-HER2 fusion (40 μg). Mice were analyzed after 3 weeks and results are shown as pictures of tumors-invaded lungs (1 representative lung per group) and in the graph below expressed as percent of lung surface invaded by melanin-loaded tumor nodules. Results represent the mean ± SD of 5 mice per group of two independent experiments. **P < 0.005 versus control, *P < 0.04 versus αGalCer. (B) Mice were grafted as above and treatment was started 6 days after with the same protocol as in (A) including treatment with sCD1d (25 μg). Lung nodules were analyzed after 2 weeks. Results represent the mean ± SD of 6 mice per group of two independent experiments. ***P = 0.0006 versus control and **P < 0.004 versus αGalCer, *P < 0.02 versus sCD1d (Stirnemann et al. 2008).*

Future development of the project by Dr. Alena Donda : In order to get closer to a clinical application, we will focus on three aspects: 1) Development of the human homologue of recombinant soluble CD1d and genetic fusion to different antibody fragments, in order to extend the targeting of CD1d to different type of cancer (anti-HER2 targeting breast cancer; anti-CEA targeting colon carcinoma). 2) Further analysis of the molecular mechanism allowing the sustained iNKT cell activation by αGalCer loaded on soluble CD1d molecules, as compared to the short lived activation induced by free αGalCer. 3) Development and exploitation of the effect of sustained iNKT cell activation on the adaptive immune response. In particular, the influence of the tumor-targeted CD1d treatment on T cell crosspriming by tumor antigens will be evaluated, taking as examples the xenoantigens HER2 and CEA, as well as different human TAA already used in active T cell immunotherapy of cancer patients. In parallel, the expected synergic antitumor effect of the continuous activation of iNKT and NK cells and an active antitumor immunization will be investigated in two models.

Dotto Gian-Paolo, Professor

Gian-Paolo Dotto received his MD from the University of Turin, Italy, in 1979, and his PhD in genetics from the Rockefeller University, New York, in 1983. After postdoctoral training at the Whitehead Institute/MIT in Cambridge, Mass., in 1987 Dr. Dotto joined Yale University, New Haven, Connecticut, as assistant professor of Pathology. In 1992 he was promoted to the rank of associate professor and soon after moved to Harvard Medical School, as associate professor of Dermatology in the newly established Cutaneous Biology Research Center. In 2000 he was promoted to the rank of Professor at Harvard Medical School and Biologist at Massachusetts General Hospital. In 2002 he accepted a position of Professor in the Department of Biochemistry at the University of Lausanne.

Human epithelial stem cell signaling and carcinogenesis

Cancer development results from deregulated control of stem cell populations and alterations in their surrounding environment. Notch signaling is an important form of direct cell-cell communication involved in cell fate determination, stem cell potential and lineage commitment. The biological function of this pathway is critically context-dependent. In our laboratory we are focusing on the pro-differentiation role and tumor suppressing function of this pathway in keratinocytes and skin, downstream of p53 and in cross-connection with other determinants of stem cell potential and/or tumor formation, like p63, EGFR and Rho/CDC42 effectors. Our most recent work points to the possibility that Notch signaling elicits a duality of signals, involved in growth/differentiation control as well as cell survival, with important implications for novel approaches of cancer therapy.

Self-renewal of human epithelial stem cells versus their commitment to differentiation and/or cancer development are closely linked. Understanding this process is of great potential impact for new therapeutic approaches to human tumors, which are mostly of epithelial origin.
Keratinocytes provide an attractive experimental system to study the connection between growth/differentiation potential of epithelial cells and transformation. Over the last few years we have been focusing on the role of the Notch signaling pathway in keratinocyte growth/differentiation control and tumor development (Dotto, 2008; Lefort and Dotto, 2004).
Notch signalling is an important form of intercellular communication and plays a key role in cell-fate determination and differentiation. The biological function of this pathway is critically dependent on context-specific interactions with other signalling pathways. In many mammalian systems, Notch signaling enhances stem cell potential and suppresses differentiation, while in others, notably keratinocytes, it exerts an opposite function (Lefort and Dotto, 2004).
Our main working hypothesis is that, at the basis of the context-dependent function of Notch in keratinocytes, there are three main determinants of specificity : a) a cross-talk of Notch signaling with cell-type specific regulatory molecules; b) cell-type specific cross-talk of Notch with other regulatory pathways, which are themselves not cell type specific; c) cell type- and tissue-specific global organization, to which Notch contributes and in the context of which it functions.
In our previous work, with mouse primary keratinocytes and skin, we have established important interconnections between Notch signaling and other pathways with a significant role in this cell type, shown that these interactions occur in a keratinocyte-specific manner and provided underlying biochemical mechanisms (Devgan et al., 2005; Mammucari et al., 2005; Nguyen et al., 2006; Okuyama et al., 2004; Rangarajan et al., 2001). Our ongoing work is based on the insights and information that we have gained so far, with a shift of focus from mouse to human keratinocytes. In fact, significant differences are known to exist between growth control of cells of mouse versus human origin, including, as we have found in keratinocytes, their response to Notch activation (Nguyen et al., 2006).

Group members 2006-2007

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Over the last 2-3 years, our laboratory has been responsible for the following main findings :

Cross-regulation between Notch and p63 in keratinocyte commitment to differentiation (Nguyen et al., 2006)

While Notch signaling promotes commitment of keratinocytes to differentiation, p63, a transcription factor of the p53 family, has been implicated in establishment of the keratinocyte cell fate and/or maintenance of epithelial self renewal. Furthermore, this gene is over-expressed in a variety of epithelial tumors including oral and skin squamous cell carcinomas. p63 is expressed in proliferating keratinocytes of the basal layer of the epidermis and hair follicles, and in the basal layers of the mammary gland and the prostate, while it is strongly down modulated with differentiation. The molecular basis for control of p63 expression is not known. Similarly, while elevated p63 expression can suppress differentiation, the underlying mechanisms have not been defined.

In our work we found that p63 expression is under Notch control in both mouse and human keratinocytes (Nguyen et al., 2006). Specifically, p63 expression is negatively regulated by Notch activation through a mechanism independent of cell cycle withdrawal and involving modulation of genes in the interferon response pathway. In turn, p63 counteracts the ability of Notch1 to promote the irreversible versus reversible commitment to differentiation, through selective modulation of Notch-dependent transcription. Thus, as illustrated in Fig. 1, a cross-talk between Notch and p63 is involved in the balance between keratinocyte self renewal and differentiation.

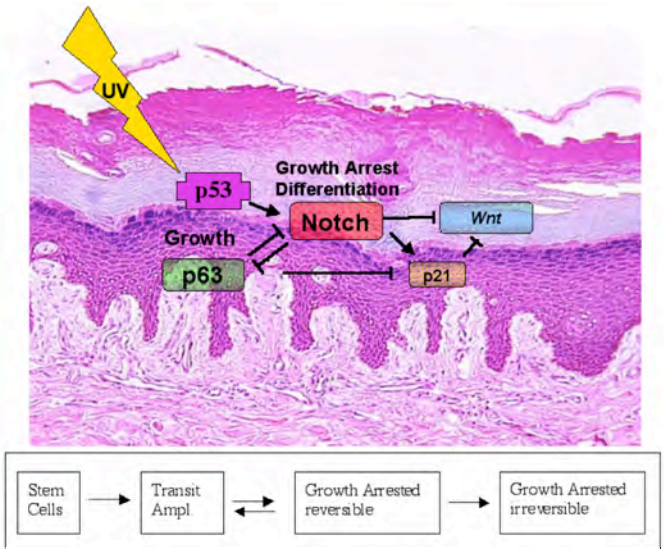

***Figure 1**: Dynamic cross-regulation of Notch with other key signaling pathways in keratinocyte stem self renewal, differentiation and/or tumorigenesis. An inverse gradient of p63 expression versus Notch activity exists in the lower versus upper epidermal layers that results from their reciprocal negative regulation. p63 exerts a dual function in suppressing Notch signaling in epidermal cells with high self-renewal potential, while synergizing with other aspects of Notch function in early stages of differentiation. Notch1 and $p21^{WAF1/Cip1}$, a "canonical" Notch target in keratinocytes, suppress* Wnt *ligand expression and signaling, and function as negative regulator of stem cell potential and tumorigenesis. In fact, UV light exposure is a major ethiological agent of human skin cancer, and the Notch1 gene is a p53 target with a key role in human keratinocyte tumor suppression.*

Referred Publications

- Dotto, G. (2008) Notch tumor suppressor function. Oncogene in press.
- Kolev, V., Mandinova, A., Guinea-Viniegra, J., Hu, B., Lefort, K., Lambertini, C., Neel, V., Dummer, R., Wagner, E.F. and Dotto, G.P. (2008) EGFR signaling as negative regulator of Notch1 gene transcription and function in proliferating keratinocytes and cancer. Nature Cell Biology in press.
- Mandinova, A., Lefort, K., Tommasi di Vignano, A., Stonely, W., Ostano, P., Chiorino, G., Iwaki, H., Nakanishi, J. and Dotto, G.P. (2008) The FoxO3a gene is a key negative target of canonical Notch signalling in the keratinocyte UVB response. The EMBO journal 27, 1243-1254.
- Lefort, K., Mandinova, A., Ostano, P., Kolev, V., Calpini, V., Kolfschoten, I., Devgan, V., Lieb, J., Raffoul, W., Hohl, D., *et al.* (2007) Notch1 is a p53 target gene involved in human keratinocyte tumor suppression through negative regulation of ROCK1/2 and MRCKalpha kinases. Genes & development 21, 562-577.
- Nguyen, B.C., Lefort, K., Mandinova, A., Antonini, D., Devgan, V., Della Gatta, G., Koster, M.I., Zhang, Z., Wang, J., di Vignano, A.T., *et al.* (2006) Cross-regulation between Notch and p63 in keratinocyte commitment to differentiation. Genes Dev 20, 1028-1042.
- Mammucari, C., Tommasi di Vignano, A., Sharov, A.A., Neilson, J., Havrda, M.C., Roop, D.R., Botchkarev, V.A., Crabtree, G.R. and Dotto, G.P. (2005) Integration of Notch 1 and calcineurin/NFAT signaling pathways in keratinocyte growth and differentiation control. Dev Cell 8, 665-676.
- Devgan, V., Mammucari, C., Millar, S.E., Brisken, C. and Dotto, G.P. (2005) p21WAF1/Cip1 is a negative transcriptional regulator of Wnt4 expression downstream of Notch1 activation. Genes Dev 19, 1485-1495.
- Lefort, K. and Dotto, G.P. (2004) Notch signaling in the integrated control of keratinocyte growth/differentiation and tumor suppression. Semin Cancer Biol 14, 374-386.

Notch1 is a p53 target gene involved in human keratinocyte tumor suppression (Lefort et al., 2007)

An inverse relationship exists between induction of differentiation and tumor development. Consistent with its role in maintaining lymphocyte stem cell populations, the role of Notch signaling in promoting human T cell leukemia is by now well demonstrated. A proto-oncogenic function of this pathway in human breast and ovarian carcinogenesis and in melanoma progression is also emerging. By contrast, the possibility that Notch signaling plays an alternative tumor suppressing function in human cancers of other type, where it can promote differentiation, has only been suggested on the basis of activated Notch1 over-expression studies. In our recent work, we have shown that Notch1 gene expression and activity are substantially down-modulated in keratinocyte cancer cell lines and tumors, with expression of this gene being under positive control of the p53 tumor suppressor in these cells. Genetic suppression of Notch signaling in primary human keratinocytes is sufficient, together with an activated *ras* oncogene, to cause aggressive squamous cell carcinoma formation (Fig. 2). Similar tumor promoting effects are also caused by *in vivo* treatment of mice, grafted with keratinocytes expressing oncogenic *ras* alone, with a pharmacological inhibitor of endogenous Notch signaling. These effects are linked with a lesser commitment of keratinocytes to differentiation, an expansion of stem cell populations and a mechanism involving effectors of small Rho GTPases, which were previously implicated in neoplastic progression.

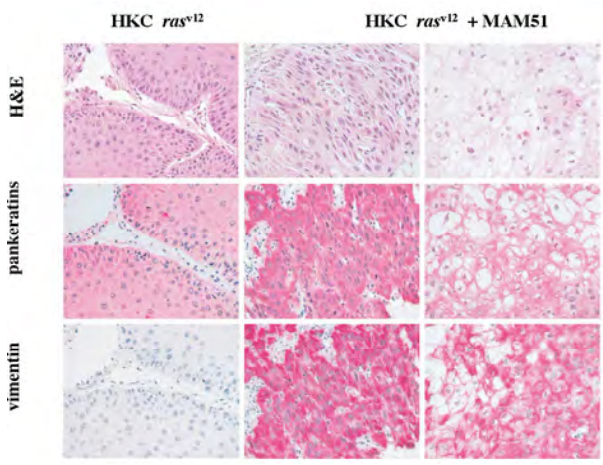

***Figure 2**: Malignant tumorigenic conversion of human primary keratinocytes by suppression of Notch signaling. To evaluate the functional consequences of decreased Notch signaling, primary human keratinocytes were infected with a retroviral vector expressing a 51 amino acid peptide (MAM51) which is widely used as a specific tool to block the "canonical" Notch-pathway. After sub-cutaneous injection into SCID mice, keratinocytes expressing oncogenic ras together with MAM51gave rise to consistent tumor formation, while control cells expressing oncogenic ras alone produced either no tumors or only small nodules (upper panel). The nodules formed by control keratinocytes expressing oncogenic ras alone had histological features of well differentiated squamous cell carcinomas or benign keratinized cysts. By contrast, tumors formed by ras and MAM51 expressing keratinocytes were moderately to poorly differentiated carcinomas, with areas of spindle cell transformation, as identified by positive vimentin expression (lower panels).*

Referred publications

- Okuyama, R., Nguyen, B.C., Talora, C., Ogawa, E., di Vignano, A.T., Lioumi, M., Chiorino, G., Tagami, H., Woo, M. and Dotto, G.P. (2004) High commitment of embryonic keratinocytes to terminal differentiation through a Notch1-caspase 3 regulatory mechanism. Dev Cell 6, 551-562.
- Rangarajan, A., Talora, C., Okuyama, R., Nicolas, M., Mammucari, C., Oh, H., Aster, J.C., Krishna, S., Metzger, D., Chambon, P., et al. (2001) Notch signaling is a direct determinant of keratinocyte growth arrest and entry into differentiation. Embo J 20, 3427-3436.

Protective anti-apoptotic function of Notch signaling in the keratinocyte UVB response (Mandinova et al., 2008)

UV light is a major etiological agent of skin aging and cancer. p53 plays a key role in the UV/DNA damage response of cells, controlling the decision between growth arrest and apoptosis. The recent finding that Notch1 is a p53 target gene in keratinocytes pointed to the possibility that this gene is also implicated in the UVB/DNA damage response of these cells. In fact, UVB exposures of keratinocytes, in culture as well as in the skin, induces Notch1 gene expression in a p53-dependent manner, with similar induction being caused by genotoxic DNA-damaging agents. Such regulation is physiologically relevant, as analysis of mice with a keratinocyte-specific deletion of the Notch1 gene as well as keratinocytes with Notch suppression showed that this gene plays a significant protective function against UVB-induced apoptosis.

As for its role in growth/differentiation control, Notch signaling can exert either a pro- or anti-apoptotic function through multiple mechanisms that are highly cell- and context-dependent. In the keratinocyte UVB response, the pro-survival function of Notch is linked to transcriptional down-modulation of FoxO3a, a key pro-apoptotic gene. This occurs without detectable changes in PI3K/Akt-dependent phosphorylation that is a major form of FoxO3a regulation. Rather, the "canonical" Notch/HERP pathway is an important negative regulator of FoxO3a expression, through a mechanism involving binding of the HES/HERP/Tle transcription repressor complex to the FoxO3a promoter.

These findings point to a dual role of Notch in promoting keratinocyte differentiation while increasing their cell survival, with important implications for possible novel approaches of cancer therapy as discussed further below.

EGFR signaling as negative regulator of Notch1 gene transcription and function in proliferating keratinocytes and cancer (Kolev et al., 2008)

Chemical Genetics is based on the principle of using small molecular weight compounds to abrogate or enhance specific regulatory pathways, providing a powerful approach to analyze complex biological systems. Little is known of pathways involved in upstream control of Notch1 gene expression and activity. In our recent work we utilized a chemical genetics approach to address this question, and identified Epidermal Growth Factor Receptor (EGFR) as a key negative regulator of Notch1 gene expression in primary human keratinocytes, intact epidermis and skin squamous cell carcinomas (SCCs).

EGFR signaling functions in the stem cell compartment of epithelial tissues, as well as in tumors, as a "built-in" mechanism to maintain self renewal and, at the same time, suppress differentiation. Our findings point to a novel role of EGFR within this context, in negative control of Notch1 gene, through a mechanism involving transcriptional down-regulation of the p53 gene by the EGFR effector c-Jun.

EGFR has become an important target of cancer drug design, and several selective EGFR inhibitors have now been approved for clinical use. However, a substantial fraction of tumors exhibit resistance to EGFR inhibition. As part of our studies, we have found that suppression of Notch signaling in squamous carcinoma cells counteracts the differentiation inducing effects of EGFR inhibitors, while, at the same time, synergizing with these compounds in induction of apoptosis. This suggests an attractive new avenue of combination cancer therapy that may

enhance the potency of EGFR inhibitory agents on tumors, while at the same time ameliorating the toxic side effects that these compounds have on normal skin, which have been attributed, at least in part, to aberrant keratinocyte differentiation.

Ongoing Research efforts

We are currently addressing three main topics :

1) Cross-talk between Notch signaling and other pathways involved in control of human keratinocyte stem cell potential and carcinogenesis;
2) Cell-type specific regulation of Notch1 gene expression;
3) Identification of positive regulators of the Notch pathway by *Chemical Genetics* (in collaboration with the Broad Institute, Harvard and MIT).

Work is focused on primary keratinocytes of human origin, as well as on human squamous cancer cell lines and primary tumors. We are taking advantage of recent advances in genetic manipulations of these cells and of their use in a 3D organotypic culture system that recapitulates the program of vertical differentiation and epithelial-mesenchymal interactions that occur *in vivo*. In addition, we have developed organ culture conditions for maintenance of intact human skin as well as freshly excised skin tumors up to 1 week, for genetic and pharmacological manipulations. These *in vitro* approaches are complemented *in vivo* by skin reconstitution grafting assays, to assess long term stem cell potential and tumorigenesis (Fig. 3).

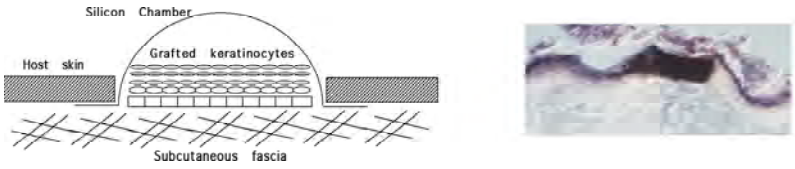

Figure 3*: The keratinocyte grafting technique and resulting formation of epidermal proliferative units (EPU) by genetically labeled keratinocytes. Cultured keratinocytes are trypsinized and injected, either alone or admixed with dermal fibroblasts, into open-bottom silicon transplantation chambers that have been placed onto the back of the mouse. Grafted cells readily attach to the underlying subcutaneous tissue and form a well differentiated epidermis by 1-2 weeks of grafting. Grafting of unlabeled and genetically labeled keratinocytes (in this case infected with an alkaline-phosphatase expressing adenovirus) allows detection of well delimited vertical epidermal proliferative units (EPUs), which are each the predicted product of individual progenitors or stem cells).*

Nicolas Fasel, Associate Professor

Nicolas Fasel is an associate professor at the Faculty of Biology and Medicine of the University of Lausanne. After studying biology at the University of Fribourg (Switzerland) and obtaining a doctoral degree at the Swiss Institute for Experimental Cancer Research working on mouse mammary tumor virus, he took up a post-doctoral position at the University of California Los Angeles working on immunoglobulin gene regulation. On his return to Switzerland, he studied post-translational modifications of cell surface antigens. As an independant researcher of the Dr. Max Cloëtta Research Foundation, he had the opportunity to establish his own group investigating the molecular and cellular biology of protozoan parasites. Since September 2006, he is director of the Department.

Group members 2006-2007

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Monica Rengifo, Trainee

Functional studies of virulence factors in the human protozoan parasite *Leishmania*

The human parasite Leishmania is the causative agent of leishmaniasis, one of the most important human protozoan diseases. Leishmaniasis may lead, depending on the species, to self-healing cutaneous lesions, mucocutaneous lesions, or fatal generalised visceral infection. Currently, the world-wide prevalence of leishmaniasis is 12 million cases, with 350 million people living in high risk areas. With the spread of the HIV epidemy to areas that are traditionally endemic for leishmaniasis, together with the emergence of drug resistant parasites, the migration of populations to endemic regions and climate changes, a dramatic increase in the number of Leishmania infections has been reported. Leishmania protozoan parasites differentiate from non- infective to infective promastigote forms in the alimentary tract of the sandfly vector, and then transform into non-motile intracellular amastigotes in host macrophages. These transformations are necessary for the successful transmission of the parasite into the host. Understanding the molecular molecular mechanism governing survival and dissemination of the parasite in the host will lead to the identification of new target molecules which could be used in chemotherapy and vaccine development, thus contributing significantly to the control of leishmaniasis in the Old and New World.

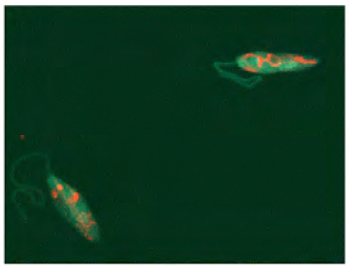
In recent years, our contribution to *Leishmania* pathogenicity was mainly aimed at the characterization of the importance of cell death for Leishmania parasites (Part A), on the identification of virulent markers in New World *Leishmania* of the *Viannia* subgenus (Part B) and more recently on the response of the host to parasites harboring a metastatic phenotype (Part C).

(A) Cell death in Leishmania parasites

Human protozoan parasites such as *Plasmodium* and trypanosomatids in particular, exhibit several features suggestive of the presence of a pathway leading to cellular death (CD) upon exposure to anti-parasitic drugs, stress factors and differentiation. However, the existence of such a pathway is based mainly on the description of morphological features and requires further characterization. In the course of our studies on gene expression during the life cycle of the parasite, we observed a DNA fragmentation pattern at specific stages of the parasite life cycle, reminiscent of cells undergoing apoptosis. Our recent work demonstrated that under a variety of stress conditions such as serum deprivation, heat shock and nitric oxide treatment, cell death could be induced activating specific proteases. Our data are consistent with the presence of metacaspase, a caspase-related protease, which has been found in plants, fungi, yeast and protozoa. The significant sequence and structural similarities with caspases suggest that metacaspases could play a similar role to caspases in programmed cell death (PCD).

The *Saccharomyces cerevisae* metacaspase YCA-1 has been implicated in the death of aging cells, cells defective in some biological functions, and cells exposed to different environmental stresses. We therefore studied the functional heterologous complementation of a *S. cerevisae yca-1* null mutant with the *L. major* metacaspase (LmjMCA) in CD induced by oxidative stress. We showed that LmjMCA is involved in yeast CD similarly to YCA-1 and that this function depends on its catalytic activity.

LmjMCA was found to be auto-processed as caspases, however, LmjMCA did not exhibit any activity with caspase substrates. In contrast, LmjMCA was active towards substrates with arginine. LmjMCA was more active when its purified processed form was tested and was found to lose its activity when the amino acids in the catalytic dyad were changed to alanine. Our results demonstrated that LmjMCA plays a similar role to YCA1 in yeast PCD and will thus allow the evaluation of the role of metacaspase in trypanosomatid PCD. In addition, the determination of the substrate specificity was useful to determine the activity of a related caspase, the paracaspase which is present in higher eukaryotes (cf. Prof. M. Thome-Miazza). Thus, there is a growing interest in the enzymatic and functional characterization of these ancient caspases not only in unicellular but also in higher organisms.



Cellular localization of LmjMCA (green) and mitochondrial staining (red)

Structurally, LmjMCA is characterized by the presence of an N-terminal mitochondrial localization signal and a proline-rich C-terminus flanking the catalytic domain. We are currently characterizing LmjMCA processing sites and subcellular localization. By biochemical fractionation and confocal microscopy, we showed that LmjMCA is present in the cytoplasm and in the mitochondrion and could be differentially processed and localized in the life-cycle or upon H2O2 treatment. Furthermore, over-expression of LmjMCA enhances susceptibility of parasites to oxidative stress. This differential expression is likely to be important in the interaction of the metacaspase with its hypothetical substrates and in its physiological role in the parasite. (In collaboration with Prof. Jeremy Mottram, University of Glasgow).

(B) Identification of virulent markers in Leishmania Viannia species

Parasites of the *Leishmania Viannia* subgenus are major causative agents of mucocutaneous leishmaniasis (MCL), a disease characterized by parasite dissemination (metastasis) from the original cutaneous lesion to form debilitating secondary lesions in the nasopharyngeal mucosa. Little is known about the pathogenesis of mucocutaneous leishmaniasis, especially about the dissemination of the infection from the site of inoculation to secondary sites (metastasis). The identification and characterisation of molecular factors associated with virulence and of markers for metastasizing subspecies of the *L. braziliensis* complex will contribute significantly to understanding the progression of the disease, as well as provide tools for the early detection of this virulence trait and the prevention of metastatic manifestations.

We employed a protein profiling approach to identify potential metastasis factors in laboratory clones of *L. (V.) guyanensis* with stable phenotypes ranging from highly metastatic (M+) through to infrequently metastatic (M+/M-) and non-metastatic (M-).

Selected publications

- Ambit, A., Fasel, N., Coombs, G. and Mottram, J. (2008) An essential role for the Leishmania major metacaspase in cell cycle progression. Cell Death Differ. 15, 113-122.
- Rebeaud, F., Hailfinger, S., Posevitz-Fejfar, A., Tapernoux, M., Moser, R., Rueda, D., Gaide, O., Guzzardi, M., Iancu, E.M., Rufer, N., Fasel, N. and Thome, M. (2008) The proteolytic activity of MALT1 is key to T-cell activation. Nat. Immunol. 9, 272-281.
- Masina, S., Zangger, H., Dutoit, V., Delgado, M.B. and Fasel, N. (2007) Histone H1 regulates chromatin condensation and infectivity of *Leishmania* parasites Experimental Parasitology 116, 83- 87.
- Moreno, J., Nieto, J., Masina, S., Canavate, C., Cruz, I., Chicharro, C., Carrillo, E., Napp, S., Reymond, C., Kaye, P., Smith, D., Fasel, N. and Alvar, J. (2007) Immunisation with H1, HASPB1 and MML *Leishmania* proteins in a vaccine trial against experimental canine leishmaniasis. Vaccine 25, 5290-5300.
- González, I.J., Desponds, C., Schaff, C., Mottram, J.C. and Fasel, N. (2007) *Leishmania major* metacaspase can replace yeast metacaspase in programmed cell death and has arginine-specific cysteine protease activity. Int. J. Parasitology 37, 161-172.
- Walker, J., Acestor, N., Saravia, N. and Fasel, N. (2006) Identification of Protein Factors associated with Virulence of Leishmania Viannia Using Proteomics Approaches. Mol. Biochem. Parasitol. 145, 254-64.
- Acestor, N., Masina, S., Ives, A., Walker, J., Saravia, N. and Fasel, N. (2006) Resistance to oxidative stress is associated with metastasis in mucocutaneous leishmaniasis J. Inf. Diseases 194, 1160-1167.

Comparison of the soluble proteomes of promastigotes by two-dimensional electrophoresis revealed that (M+) and (M-) clones express distinct acidic and neutral isoforms of cytosolic peroxiredoxin (TXNPx). We examined in more detail the functional relationship between cytoplasmic peroxiredoxin and metastatic phenotype using high and low metastatic variant clones of a *Leishmania (V.) guyanensis* strain previously shown to be highly metastatic in golden hamsters. We concluded that distinct forms of cytoplasmic peroxiredoxin were found to be associated with the metastatic phenotype. We reported that peroxidase activity in the presence of hydrogen peroxide and infectivity differs between metastatic and non-metastatic *Leishmania Viannia guyanensis* clones. Upon hydrogen peroxide treatment or heat shock, peroxiredoxin was detected preferentially as dimers in metastatic *Leishmania (V.) guyanensis* clones and in *Leishmania Viannia panamensis* strains from MCL patients, when compared to non-metastatic parasites. These data provided evidence that resistance to the first microbicidal response of the host cell by *Leishmania* promastigotes is linked to peroxiredoxin conformation and may be relevant to intracellular survival and persistence, which are prerequisites for the development of metastatic disease. (In collaboration with Prof. Nancy Saravia and Dr. John Walker, CIDEIM, Cali Colombia).

(C) Analysis of gene expression in macrophages infected with metastatic and non metastatic *L. guyanensis*

(under the supervision of Dr. S. Masina)

Infections with *L. (V.) guyanensis* parasites are distinguished by their metastatic dissemination from the original skin lesions leading to the production of secondary mucocutaneous lesions in the naso-pharyngeal tissues of the infected host. Metastatic dissemination is poorly understood and mucosal disease is difficult to treat. cDNA microarrays were used to identify differentially expressed macrophage genes implicated in host parasite interaction, which in turn provide valuable leads for future studies toward the identification of candidate drug targets for immuno-prophylactic or therapeutic intervention. As mentioned previously, in the hamster model of *L. (V.) guyanensis* infection, different clones of the parasite have been isolated and characterized as either metastatic or non-metastatic (Martinez *et al.* 2000). To understand the mechanism by which *L. (V.) guyanensis* parasites cause MCL in the host, we used representative metastatic (M+) and non-metastatic (M-) clones to intracellularly infect host macrophages and examine the effect on host cell gene expression using 17K mouse cDNA microarrays by screening with RNA obtained from mouse bone marrow macrophages infected or uninfected with (M+) or (M-) parasites. The comparison of bone marrow macrophages (BMM∅'s) infected with (M-) versus BMM∅'s infected with (M+) parasites was the most interesting in our hands. Of particular interest was the obtained list of 82 genes when all three biological experiments were compared.

We can speculate that the pattern of genes up or down-regulated by the metastatic clone optimizes the chances of survival and dissemination of this parasite leading to MCL. We found in the list of 82 genes, some candidates that are suspected to play a role in parasite virulence and propagation.

Sanjiv Luther, Assistant Professor

Sanjiv Luther studied cell biology at the ETH in Zuerich. He received his PhD in 1996 from the University of Lausanne for his work on anti-viral immune responses in the laboratory of Hans Acha-Orbea. He then moved to the laboratory of Jason Cyster at the Howard Hughes Medical Institute at the University of California San Francisco where he investigated the role of chemotactic factors in lymphoid tissue development and function. In August 2003 he joined the Department of Biochemistry as a Swiss National Science Foundation Assistant Professor.

Group members 2006-2007

Stéphanie Favre, Technician
John Perrin, Technician
Mirjam Britschgi, PhD student
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Stefanie Siegert, PhD student
Tobias Vogt, PhD student

Lymphoid tissue stromal cells in health and disease

Secondary lymphoid tissues, such as lymph nodes and spleen, are the only sites where immune responses against pathogens are efficiently initiated. It is within the T cell rich zone of these organs that dendritic cells present the captured pathogens to recirculating T cells in order to activate the rare antigen-specific T cells. While we have made considerable progress in understanding the biology of dendritic cells and T lymphocytes, we know very little about the stromal cells that form the 'niches' within this unique microenvironment. Over the last four years we have developed the technology to isolate, culture and characterize stromal cells of the T zone at the phenotypic and functional level. We could demonstrate that these cells are indeed critical for efficient T cell homeostasis and activation. The interest of the lab is now focused on dissecting further the development, phenotype and function of these poorly characterized T zone stromal cells, both during homeostasis and disease.

Stromal cells of secondary lymphoid organs

Stromal cells in primary lymphoid organ, such as the bone marrow and thymus, have been extensively studied and shown to be important for the generation of niches allowing the correct localization, differentiation and proliferation of haematopoietic cells. Besides providing a structural framework, they do so by providing several factors such as cytokines, chemokines, adhesion molecules and extracellular matrix proteins.

Much less is known about stromal cells in secondary lymphoid organs. In the last few years it has become clear that the compartmentalization of these organs into B and T cell-rich zones is achieved by specialized resident stromal cells which constitutively produce chemotactic factors (chemokines). B zone stromal cells (also referred to as follicular dendritic cells or FDC) have been characterized phenotypically and functionally and attributed several important functions for B cells, including the production of the B cell attractant CXCL13.

However, their T zone counterpart, called fibroblastic reticular cells (FRC), has been mainly characterized in tissue sections. They resemble fibroblasts, associate with collagen fibres and can be selectively stained using the marker gp38. For a long time FRCs were thought to be mainly responsible for the structural stability of the T zone. In the year 2000 we proposed a more active function for gp38+ T zone stromal cells based on the evidence that a stromal cell type in the T zone represents the major constitutive source of the chemokines CCL19 (ELC) and CCL21 (SLC). These chemokines share the same receptor CCR7 and efficiently attract CCR7-bearing dendritic cells and T cells. The importance of T zone stromal cells and the CCR7 ligands most likely secreted by them is best illustrated in a natural mouse mutant (*plt/plt*) that we and others have

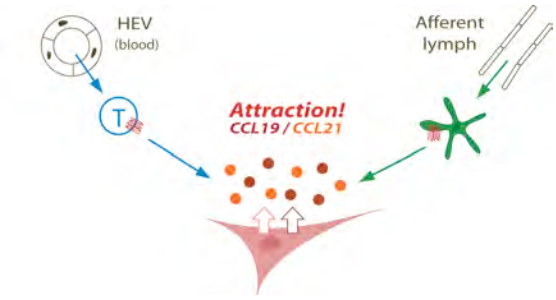

***Figure 1**: T zone stromal cells (in red) produce CCL19/21 and thereby attract CCR7+ T cells (in blue) and DCs (in green) into T zones allowing their physical interaction.*

Selected publications

- Scandella, E., Bolinger, B., Lattmann, E., Miller, S., Favre, S., Littman, D., Finke, D., Luther, S.A., Junt, T. and Ludewig, B. (2008) Restoration of lymphoid organ integrity through interaction of lymphoid tissue inducer cells with the T cell zone stroma. Nature Immunol. 9, 667-675 (cover story). Commentary on this article in Nature Reviews in Immunology 8, 400 (2008).
- Link, A., Vogt, T.K., Favre, S., Britschgi, M.R., Acha-Orbea, H., Hinz, B., Cyster, J.G. and Luther, S.A. (2007) Fibroblastic reticular cells in lymph nodes regulate naïve T cell homeostasis. Nature Immunol. 8 (11), 1255-65. Commentary on this article in Nature Reviews in Immunology 7 (11), 839.
- Luther, S.A., Serre, K., Cunningham, A.F., Khan, M., Acha-Orbea, H., MacLennan, I.C.M. and Toellner, K.M. (2007) Recirculating CD4 memory T cells mount rapid secondary responses without major contributions from follicular CD4 effectors and B cells. Eur. J. Immunol. 37, 1476-84.
- Lang, K.S., Recher, M., Junt, T., Navarini, A.A., Harris, N.L., Freigang, S., Odermatt, B., Conrad, C., Ittner, L.M., Bauer, S., Luther, S.A., Uematsu, S., Akira, S., Hengartner, H. and Zinkernagel, R.M. (2005) Toll-like receptor engagement converts T cell autoreactivity into overt autoimmune disease. Nature Medicine 11 (2), 138-145.

described to lack both CCR7 ligands within lymph nodes and spleen. In *plt/plt* mice dendritic cells and T cells fail to accumulate in the T zone leading to an inefficient induction of T cell activation.
More recently, FRCs were shown to form a 3-dimensional network to which dendritic cells adhere. Interestingly, migrating T cells use this network as a road system for their continuous migration leading them past the antigen-presenting dendritic cells. Therefore, they are thought to improve the efficiency of immune response induction by bringing antigen-specific T cells together with the antigen-presenting cells. Despite this recent progress a detailed study of FRC was not possible due to the lack of appropriate cell isolation techniques.

Phenotype of T zone stromal cells in lymph nodes

Our histological analysis of lymph nodes revealed that gp38 expression is not restricted to FRC in T zones. Lymphatic endothelial cells in lymph nodes co-express gp38 and CD31 while B zone stromal cells upon activation coexpress gp38 and CD35.
When we tested various cell isolation techniques based on enzymatic digestion of lymph nodes we found that a one-hour-long collagenase digestion released best gp38+CD31-CD35-CD45- cells. These cells had a distinct surface phenotype, including the expression of LTβR and TNF-R1 which are thought to act upstream of CCL19/21 expression. Indeed, sorted FRC expressed very high levels of CCL19 and CCL21 transcripts when compared to 5 other cell populations in LN. Thus, we have developed the first protocol allowing the isolation and *ex vivo* characterization of CCL19/21+ FRCs. The coexpression of both PDGFR chains (αβ) suggested a mesenchymal origin for these cells. As we observed desmin and α-SMA expression within these cells, their contractile capacity was tested (in collaboration with Boris Hinz, EPFL). Indeed, these cells were able to contract efficiently a thin silicone substrate suggesting they are functional myofibroblasts.

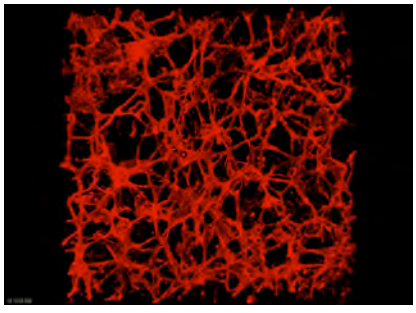

***Figure 2**: Gp38+ T zone stromal cells form a dense network of fibroblast-like cells throughout the T zone of lymph nodes (3D reconstruction of a vibratome section).*

Function of T zone stromal cells in lymph nodes

Our demonstration of CCL19 and CCL21 production by FRC is clear evidence for an important role of these cells in adaptive immunity given that *plt/plt* mice show a defect in T cell priming. When looking for novel functions of FRC we considered its strategic position in the T zone where T cells spend hours migrating along the FRC network. Recirculating T cells are known to depend on the cytokine IL-7 for survival; however, the organ and cells providing this factor were not known. In situ hybridization analysis revealed a reticular pattern of IL-7 expressing cells throughout the T zone of LNs and realtime PCR on cDNA of sorted LN cells showed the highest IL-7 expression within FRCs. In cell culture FRC were the only cell type able to keep naïve CD4+ and CD8+ T cells alive over

several days. Surprisingly, this effect was only partially due to IL-7. The second signal proved to be pertussis-toxin sensitive and mainly due to CCL19. Recombinant CCL19 alone was sufficient to keep naïve T cells alive although to a lesser extent than recombinant IL-7. As an in vivo test for the role of LNs and FRCs in naïve T cell homeostasis, we blocked LN access for the transferred naïve T cells by interfering with critical integrin or chemokine signals (collaboration with Hans Acha-Orbea, Lausanne). Indeed, such interference led to a similar decrease in peripheral T cell numbers as blocking the survival signal IL-7 itself. To assess the potential role of CCL19 in T cell survival in vivo, wildtype T cells were transferred into CCL19-/- mice which show a normal lymphoid tissue organization (collaboration with Jason Cyster, San Francisco). In a CCL19-deficient environment, naïve T cells disappeared more rapidly than in wildtype mice indicating its non-redundant role in naïve T cell homeostasis. In summary, recirculating T cells appear to 'recharge their batteries' during their several hour long stay within T zones by collecting survival factors such as IL-7 and CCL19 produced by TRCs. Secondary lymphoid organs and in particular FRCs within the LN T zone control the size of the peripheral T cell pool and thereby the available repertoire for an adaptive immune response. Therefore, FRCs are not just structurally important cells but play a central and very active role in adaptive immunity.

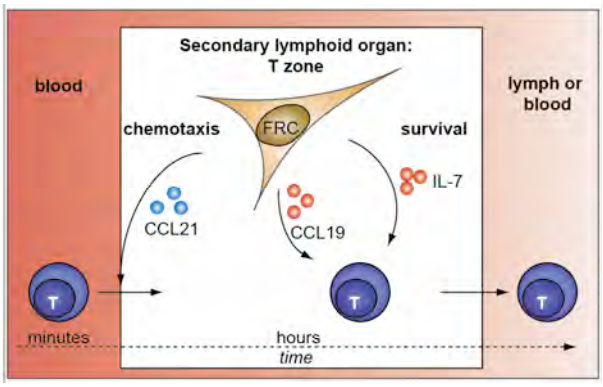

***Figure 3**: Model showing the central role FRC play for recirculating T cells. On the one hand they attract T cells by producing the chemoattractants CCL19 and CCL21. On the other hand they provide them with survival factors, such as IL-7 and possibly CCL19.*

Function of T zone stromal cells in disease

In collaboration with the laboratory of B. Ludewig (St.Gallen) we have characterized lymphoid tissue stromal cells during LCMV-WE infection. GP38+ FRCs in spleen were destroyed 8-12 days after infection due to FRC infection by LCMV and their elimination by CD8+ T cells. This destruction led to the disappearance of distinct white pulp cords and was associated with a strong immunodeficiency towards a subsequent VSV infection. Surprisingly, the stromal cell network reappeared within few days leading to white pulp formation and return of immunocompetence. This reconstruction phase depended on lymphoid tissue inducer cells (LTi) that started to proliferate during the phase of acute immunosuppression. Therefore, LTi cells come into play during lymphoid tissue injury and recapitulate processes involved in lymphoid tissue genesis. A key target of LTi cells are presumably the stromal cells which act as tissue organizer cells.

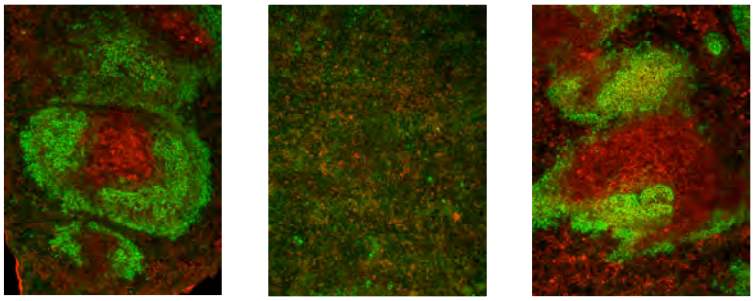

Figure 4*: Stromal cell networks of the splenic white pulp on day 0 (left), 12 (middle) and 25 (right) after LCMV-WE infection. CD35+ B zone stroma (in green) and gp38+ T zone stroma (in red) are destroyed completely on day 12 and reconstructed few days later.*

Perspective

Given that we can now isolate, culture and characterize FRC at the single cell level opens up many avenues of future research. Our present and future studies aim at improving our understanding of the T zone stromal cells and their role for T cells and dendritic cells in homeostasis and immune response. Importantly, lymphoid infiltrates observed at sites of inflammation or cancer are associated with similar stromal cell networks. Therefore our studies should help in designing intervention strategies to enhance beneficial or suppress harmful immune responses within secondary or tertiary lymphoid tissues by targeting directly the organizer cells, such as the T zone stromal cells.

Current collaborators

Hans Acha-Orbea (Univ. of Lausanne), Jeff Browning (Biogen, Boston), Chris Buckley (Univ. of Birmingham, UK), Jason Cyster (UCSF, San Francisco), Daniela Finke (Univ. of Basel), Matthias Heikenwaelder (Univ. Hospital, Zuerich), Boris Hinz (EPF Lausanne), Lukas Kuehn (ISREC, Lausanne), Dan Littman (NYU, New York), Burkhard Ludewig and Elke Scandella (Kantonal Hospital of St.Gallen), Rob MacDonald (LICR, Lausanne), Ian MacLennan and Kai Toellner (University of Birmingham, UK), Michael Sixt (MPI Munich), Charles Surh (Scripps Clinics, San Diego), Melody Swartz (EPF Lausanne) and Carl Ware (LIAI, San Diego).

Andreas Mayer, Professor

Andreas Mayer studied biology and chemistry at the University of Munich. In 1995 he obtained a PhD from the same university for studies on protein translocation into mitochondria in the laboratory of Walter Neupert. After postdoctoral studies on organelle inheritance and fusion with William Wickner at Dartmouth Medical School, Andreas Mayer joined the Friedrich-Miescher-Laboratorium of the Max-Planck-Society as a group leader in 1997. In 2003 the group moved to the Department of Biochemistry of the University of Lausanne, continuing its work on the mechanism of membrane fusion and on microautophagic membrane dynamics.

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Bernd Strasser, PhD student
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Membrane fusion and the VTC complex

The research group has been studying the molecular mechanisms of vesicular transport in eukaryotic cells, focusing on the fundamental question of how intracellular membranes fuse and fragment. We have addressed the issue using yeast lysosomes (vacuoles). Factors involved in fusion and fragmentation of the organelle have been characterized by kinetic analyses, genetics, pharmacological screens, biochemical fractionation, and by cytology in vivo and in vitro. During the last two years we have made several major discoveries: First, we have identified the enzymatic activity and determined the crystal structure of the catalytic core of the VTC complex, a membrane integral complex required for vacuole fusion. Second, we have performed a genetic screen to identify proteins required for membrane fission activity that fragments the organelle into small vesicles under osmotic stress. Third, we could reconstitute the fragmentation process with purified vacuoles in vitro. Fourth, we could show that the vacuolar membrane fusion and fission machineries are intimately linked and control each other. These observations lay an excellent foundation for analyzing how an entire organelle can be cleaved into small vesicular fragments.

Membrane fusion is a basic biochemical reaction prerequisite to the compartimentation of eukaryotic cells. It is required for transport of proteins and lipids between organelles. In exocytosis it controls many vital processes, such as signal transduction among neurons, secretion of hormones or digestive enzymes, the regulation of sugar transporters, or the cytotoxic activity of T-lymphocytes. In all of these examples controlled membrane fusion is a key event for the delivery and activation of signaling molecules, cytotoxic substances, receptors etc. at the cell surface. Also uptake and persistence of intracellular parasites are closely related to vesicular trafficking. Membrane fusion reactions on all compartments of the secretory and endocytic pathway share conserved factors and follow similar reaction pathways.

Fusion of vacuoles depends on SNAREs, Rab proteins and their effectors, and on the V_0 sector of the vacuolar H^+-ATPase. SNARE activation and also lipid turnover on the vacuolar membrane depend on the VTC complex, which contains four proteins of so far unknown molecular activity. We have begun to analyze the Vtc proteins by X-ray crystallography and ligand binding studies. Hydrophilic domains of Vtc2, Vtc3, and Vtc4 were analyzed by limited proteolysis. This revealed two stable domains: the SPX domain and an approx. 35 kDa middle domain. All were expressed in E. coli, purified and crystallized and structures were determined in collaboration with the groups Klaus Scheffzek (EMBL). The Vtc2 middle domain was solved at 2.5 Angstroms and the Vtc4 middle domain at 2.9 Angstroms. Extensive substrate binding studies were performed with both, suggesting a high affinity for nucleotide triphosphates. We obtained co-crystals of the Vtc4 central domain with its ligand ATP and with an enyzmatic product, polyphosphate (Fig. 1). These studies thus identified the VTC complex as the first eukaryotic polyP polymerase and provided information about the structure of its catalytic domain. The structures indicate a novel mode of ATP binding and potentially a novel mode of ATP hydrolysis.

Plants, fungi and unicellular organisms can store large quantities of phosphate as polyphosphate (polyP) which can be mobilized under conditions of phosphate and energy limitation. For some organisms, such

Selected publications

- Apel, A., Herr, I., Schwarz, H., Rodemann, H.P. and Mayer, A. (2008) Blocked Autophagy Sensitizes Resistant Carcinoma Cells to Radiation Therapy. Cancer Res. 68, (5).
- Uttenweiler, A. and Mayer, A. (2008) Microautophagy in the Yeast Saccharomyces cerevisiae. Methods in Mol. Biol. 445, 245-259.
- Baars, T., S. Petri, C. Peters and A. Mayer (2007) Role of the V-ATPase in regulation of the vacuolar fission-fusion equilibrium. Mol. Biol. Cell 18, 3873-82.
- Uttenweiler, A., Schwarz, H., Neumann, H. and Mayer, A. (2007) The vacuolar transporter chaperone (VTC) complex is required for microautophagy. Mol. Biol. Cell. 18, 166-175.

as the parasites Trypanosoma or Leishmania, this ability is crucial for survival. Vtc proteins exist in virually all unicellular eukaryotic species, but not in plants or animals. Since the VTC proteins are crucial for viability of parasites but apparently absent from their hosts they may even represent a potential drug target. We identified two isoforms of the Vtc complex, one localized mainly to vacuoles, the other one localized to the plasma membrane. We propose that vacuolar VTC complexes create a vacuolar polyP phosphate store whereas the plasma membrane forms synthesize polyP into the extracellular space. In symbiotic fungi – plant interactions (mycorrhizae) this extracellular fungal polyP might serve to transfer phosphate from the fungal hyphae to the plant root.

The structure suggests that, in addition to polyphosphate and ATP, the VTC complex may bind other negatively charged ligands. This is interesting because the Vtc mutants also show defects in vacuolar lipid turnover during the in vitro fusion of vacuoles. We mutated several of the positive residues in the active center of the enzyme. The single point mutations left the complex stable and intact but eliminated lipid turnover. In addition, the VTC complex strongly accumulates at the interfaces where two vacuoles touch each other. This suggests that the VTC complex might also be involved in lipid turnover in vacuole membranes – which may explain its role in vacuole fusion. Further analysis will be required in order to resolve the links between phosphoinositide and polyphosphate metabolism.

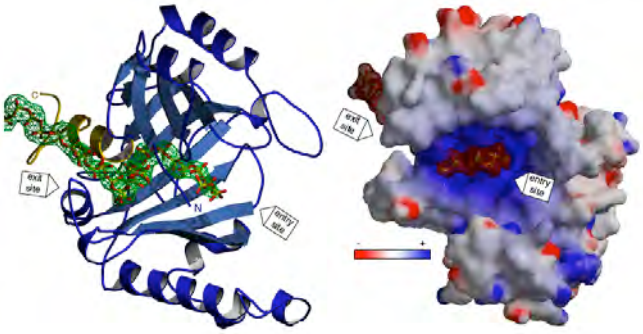

Figure 1*: Crystal structure of a Vtc protein with polyphosphate bound in the catalytic tunnel (left) and with the substrate ATP bound (right) at the entry of the tunnel.*

Vacuole fragmentation and the equilibrium of fusion and fission activities on an organelle

Many organelles exist in reasonably well-defined copy numbers and sizes in a given cell type. Both size and copy number can change in reproducible ways upon changes in environmental conditions or in response to the cell cycle. Examples include endosomes, lysosomes, peroxisomes, the Golgi matrix, mitochondria and chloroplasts. Transitions in organellar size and number are apparently controlled by the cells and it is reasonable to assume that they are the product of a regulatable equilibrium between the fundamental processes of organellar membrane fission and fusion. Yet the control mechanisms governing fission of the organelles and its coordination with the antagonistic fusion activities are largely unexplored. We want to systematically address this problem.

Organelle fission may serve different purposes, depending on the organelle. There is an apparent function in the transmission of low-copy organelles to daughter cells. For endosomes, a larger copy number of the organelle may be necessary to guarantee distribution of the organelle into different regions of the cell. Producing enough organelle copies to deposit

them in different parts of the cell may help to regulate signaling, since the location of an endosome modifies the efficiency of signaling from endocytosed receptors residing in it. For vacuoles and lysosomes there might be additional reasons for undergoing regulated cycles of fission and fusion. Vacuoles function in hydrolysis, osmoregulation and storage of amino acids and ions. During logarithmic growth a yeast cell contains 2-5 vacuoles of intermediate size. They coalesce into a single organelle upon nutrient limitation. Also hypotonic media promote vacuole coalescence. In contrast, hypertonic conditions induce fission. Vacuoles may respond in this way because fragmentation and coalescence change their surface to volume ratio. Under hypertonic conditions cells loose water. The vacuole volume is reduced but the membrane surface remains constant. Fragmentation of the organelle can readjust the surface to the reduced volume. Upon starvation, vacuoles coalesce and thus expand their volume. This parallels the strong increase in vacuolar hydrolase expression which is needed to degrade and recycle cytoplasmic material that is transferred into vacuoles by autophagy. This regulation of lysosomal hydrolytic capacity is crucial to the correct functioning of eukaryotic cells. Accordingly, mutations in genes affecting lysosomal degradation give rise to numerous lysosomal storage diseases.

Our goal is to understand how vacuole size and copy number are determined and to elucidate the interactions of the vacuole fusion and fission machineries necessary to control these parameters. This requires a detailed characterisation of the fusion and fission machineries. Characterisation of vacuole fusion is well advanced by the efforts of several laboratories. For vacuole fission, no systematic approach has been taken so far. Therefore, we have initiated two systematic approaches to the problem: The screening for vacuole fission mutants and the reconstitution of vacuole fission with purified organelles.

Screening of vacuole fission mutants

The simplest approach to obtain genes required for vacuole fission is to screen the collection of yeast deletion mutants in the 5000 non-essential genes. We stained the vacuoles in the mutant cells with the vital dye FM4-64 and subjected them to osmotic shock which induces rapid vacuole fragmentation in wildtype cells (Fig. 2). Fluorescence microscopic screening for mutants defective in this induced fragmentation identified > 100 mutants with strong phenotypes. These will be further characterized in the future.

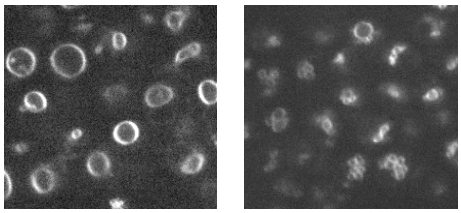

***Figure 2**: Vacuole fragmentation in vivo: Yeast cells were grown in 96 well-plates in YPD and stained with FM4-64. 5 min after the addition of 0.4M NaCl (hypertonic shock; right panel) or of water (control, left panel), the cells were analyzed in the plate by confocal microscopy. Pictures show groups of yeast cells; only the stained vacuoles are visible.*

Reconstitution of vacuole fission in vitro

Reconstitution of a complex cell-biological reaction can be very challenging and require a long time. Whereas genetic screening can identify elements of the fragmentation machinery, study of mechanism benefits from *in vitro* systems offering more facile manipulation and analysis. We succeeded in establishing a cell-free system to reconstitute fragmentation of vacuoles purified from yeast. This *in vitro* reaction reproduces the physiological characteristics of *in vivo* vacuole fragmentation: It requires the dynamin-like GTPase Vps1p, V-ATPase pump activity, cytosolic proteins, temperature, and ATP and GTP hydrolysis.

We used the *in vitro* system also as a discovery tool. Test of low molecular weight inhibitors of in vitro vacuole fission identified the drug Rapamycin as a potent inhibitor. This led us to uncover that the target of Rapamycin, TOR kinase, positively regulates vacuole fragmentation.

Control by TOR is epistatic to the stimulation of vacuole fragmentation by hypertonic stress. TOR stimulates fragmentation but not the opposing reaction of vacuole fusion. Based on these observations we can explain why vacuoles coalesce into a single organelle under nutrient restriction: Nutrient restriction inactivates TOR, thus repressing fission activity but leaving fusion active.
The in vitro system will be an extremely useful tool for future analysis of the fission mechanism. It will hence be developed further and used for characterizing the fission defects in the mutants that emanated from the genetic screen.

Interaction of the vacuolar fusion and fission machineries
Since organelle fusion and fission are antagonistic reactions one might expect mechanisms to avoid futile cycles between them and guarantee that at any given time only fission or fusion can be active, favoring fragmentation or coalescence of vacuoles, respectively. Our studies have indicated that the fission protein Vps1p, a Dynamin-like GTPase, has a negative regulatory influence on fusion. Vps1p binds the vacuolar t-SNARE Vam3p and inactivates it by preventing it from entering into trans-SNARE complexes and triggering fusion. Vam3p can be displaced from Vps1p and activated for fusion in a reaction controlled by NSF/Sec18p (Peters et al., 2004). Thus, there is negative control of a component of the fission machinery over the fusion machinery (Fig. 3). Such interactions may be of general relevance because also mammalian Dynamin, which catalyzes endocytosis, interacts with the t-SNARE Syntaxin that is involved in the opposing process of regulated exocytosis and since Dynamin can be recruited to the site of exocytosis and influence the exocytotic process.

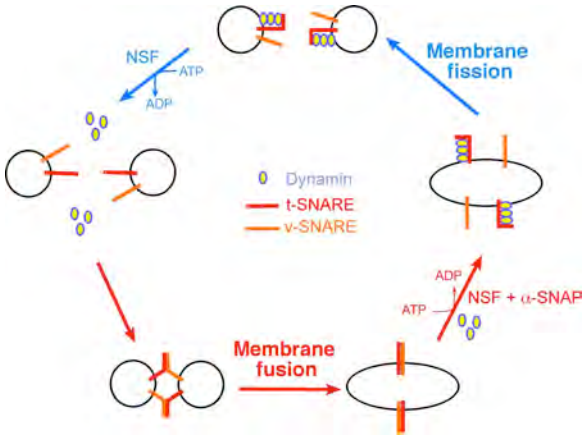

***Figure 3**: Illustration of a cycle of vacuole fusion and fission and the known interactions of the proteins at the different stages. v- and t-SNAREs form trans-complexes that trigger further events leading to fusion. After fusion, the resulting v/t cis-SNARE complexes are disrupted by the chaperone NSF and its cofactor alpha-SNAP. This reactivates the SNAREs. The separated t-SNARE binds the Dynamin-like GTPase Vps1 in its polymeric form. This interaction and the polymeric form of Vps1 promotes fission of the organelle. In addition, binding the t-SNARE to Vps1p prevents it from interacting with the v-SNARE. Thus, fusion activity is suppressed in this part of the cycle. After fission, the t-SNARE/Dynamin interaction is broken by NSF, thereby liberating the t-SNARE for forming new trans-complexes with a v-SNARE and initiating another round of fusion. At the same time, the Dynamin is released from the membranes. This ensures that only fusion is active in this part of the cycle.*

We have now addressed the question whether there is also control of the fusion machinery over the fission process. Using conditional mutants in any of the five vacuolar SNARE proteins – core components of the fusion machinery, we could show that inactivation of SNARE proteins not only blocks fusion but surprisingly also hinders fragmentation of the compartment. This indicates a regulatory influence of the fusion machinery over the membrane fission process. In sum, the machineries for membrane fission and fusion appear to be intimately coupled and to mutually influence each other. Future studies will be directed at further elucidating this complex interplay.

Pascal Launois,
Professor ad personam

Pascal Launois completed his MD in Reims, France in 1988. After specialization in Immunology and Microbiology, he joined the International Network overseas of the Institut Pasteur. He obtained his PhD in 1997 from the University Claude Bernard in Lyon, France. His postdoctoral work, conducted in the laboratories of Prof J. Louis at the University of Lausanne, focused on the early events instructing Th1 and Th2 cell development in a murine model of infection with L. major. He was appointed Director of the WHO Immunology Research and Training center in the Department of Biochemistry in May 2003.

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Mélanie Revaz-Breton, PhD student
Charles Finsterwald, Master student
Silvia Zambrana, Trainee
Patricia Terceros, Trainee

Immune responses to Leishmania infection

Mice from the majority of inbred strains (C57BL/6, C3He, Sv129…) are resistant to infection by Leishmania major (L. major), an obligate intracellular protozoan parasite of macrophages in the mammalian host. In contrast, mice from BALB strains are unable to control infection and develop progressive disease. Two subsets of $CD4^+$ T cells distinguishable by the pattern of cytokines they produce in vitro have been described. Th1 cells are characterized by secretion of IFN-γ and LT while Th2 cells produce IL-4, IL-5 and IL-13. In the murine model of infection with L. major, genetically determined resistance and susceptibility have been clearly shown to result from the appearance of parasite-specific $CD4^+$ T helper 1 (Th1) or T helper 2 (Th2) cells, respectively. This murine model of infection is considered as one of the best experimental system for the study of the mechanisms operating in vivo at the initiation of polarized Th1 and Th2 cell maturation.
Among the several factors influencing Th cell development, cytokines themselves critically regulate this process. Using this model, it has been demonstrated that IL-12 and IL-4 promote Th1 and Th2 cell development, respectively. During the last ten years, we have demonstrated that IL-4 produced by a particular subpopulation of $CD4^+$ T cells that express the Vb4 Va8 TCR chains specific for a single antigen of L. major, LACK, drives an early IL-4 response that underlies subsequent Th2 cell maturation in susceptible BALB/c mice. However, recent results showed that even if IL-4 is an important factor for Th2 differentiation, it is not the only signal necessary for Th2 differentiation in susceptible BALB/c mice.
The aim of our projects is to further investigate the immunological basis accounting for susceptibility or resistance to infection with L. major in BALB/c and C57BL/6 mice, respectively.

Group of Pr. P. Launois

The role of Toll Like Receptors (TLRs) in the shaping of protective immune response in resistant mice

TLRs are a family of receptors of the innate immunity that recognized a broad spectrum of ligands including lipids (LPS and lipoproteins), proteins (flagellin), nucleic acid and profilin. The role of TLRs in innate immune responses to virus and bacterial pathogens is widely recognized but little is known about the role of TLRs in host defenses against eukaryotic pathogens. The purpose of this project is to determine whether TLRs modulation may be induced during infection with *L. major* in susceptible BALB/c and resistant C57BL/6 mice and subsequently influences the outcome of infection by directing the development of a Th1 or Th2 response.
During the first years of this project, the results were the following:

- Using quantitative real time PCR, an up-regulation of TLR-7 and TLR-9 mRNA expression was detected during the early phase of infection (one day post infection) in draining lymph node cells of resistant C57BL/6 mice but not in susceptible BALB/c mice.
- TLR-9m RNA up-regulation was preferentially expressed in B cells.
- Genetically resistant mice (C57BL/6 background) deficient for TLR-9 ($TLR\text{-}9^{-/-}$) mice infected with *L. major* develop lager lesions than control mice and develop a Th2 cell response but at lower levels than in the corresponding susceptible mice (BALB/c). However half of $TLR\text{-}9^{-/-}$ mice resolve the cutaneous lesions.

Altogether, these results suggest that TLR-9 is not essential in the clinical cure of lesions and in the parasite control of mice infected with *L. major* LV39.

The role of B cells as APC in instructing Th 2 cell responses in BALB/c mice

Whereas B cells have been shown to induce Th2 cell differentiation, B-lymphocytes are considered to play a minimal role in host defences against *L. major*. We investigated, the contribution of B cells in susceptibility to infection with different *L. major* strains using BALB/c mice lacking mature B cells due to the disruption of the IgM transmembrane domain (μMT). Whereas BALB/c μMT are totally susceptible to infection with *L. major* IR173 and IR75, they are resistant to infection with *L. major* LV39. Adoptive transfer of naïve B cells prior infection into BALB/c μMT mice that restore susceptibility to infection confirmed the role of B cells in the susceptibility to infection with *L. major* LV39.

The two main functions of B cells are Ig secreting cells and/or APCs. Since antibodies have been shown to play a critical role in the pathogenesis associated with the infection with either *L. amazonensis* or *L. major*, we determined the role of immune serum in restoring susceptibility in BALB/c μMT mice infected with *L. major* LV39. Using different protocols of passive immune serum transfer were performed either around time of infection (day -6, -2 and +3) or during the first two weeks of infection (day +1, +7, +14) or at day 21, we were unable to restore susceptibility to infection with *L. major* LV39 in BALB/c μMT mice. Indeed adoptively transferred mice with immune serum do not develop lesion as control BALB/c mice and develop a Th1 response. In addition, whereas IL-10 produced by macrophages in response to ligation of specific antibodies to FcγR has been recently described to exacerbate disease progression, we were unable to detect IL-10 in *L. major* stimulated LN cells from BALB/c μMT mice adoptively transferred with immune serum. Thus, our present data clearly specific Ig do not contribute to susceptibility to infection with *L. major*.

The possible role of B cells as APCs is controversial. We demonstrated that, in vitro, $CD4^+$ T cells specific for LACK, a well-known antigen from *Leishmania*, proliferated in the presence of B cells. Furthermore, only antigen specific B cells account for the presentation to specific T cells. Indeed. LACK specific $CD4^+$ T cells were unable to proliferate in the presence of monoclonal B cells from HEL Tg mice that express only IgD/IgM specific for HEL – an irrelevant antigen- at the surface of B cells. Furthermore, adoptive transfer of HEL Tg B cells in BALB/c μMT mice, in contrast to adoptive transfer of normal B cells from BALB/c mice, was unable to restore susceptibility to infection with *L. major*. Interestingly, we clearly demonstrated that the inability of HEL Tg B cells to present antigen to specific T cells was not inherent to these cells but rather to the inability to internalize specific antigens. Indeed, using HEL-LACK complex, HEL Tg B cells are able to bind the HEL part of this complex trough their specific receptor, internalized it and present LACK to specific $CD4^+$ T cells. Altogether these data demonstrated the need of *Leishmania* specific B cells to induce Th2 cell development and susceptibility to infection with *L. major*. Unfortunately, using biotinylated LACK, we were unable to detect LACK specific B cells in naive B cells from BALB/c mice suggesting that the frequency of LACK specific B cells is low and undetectable using such methods.

Fabienne Tacchini-Cottier,
Associate Professor ad personam

Fabienne Tacchini-Cottier studied immunology at Stanford University, California, where she obtained her PhD. She then became "maître-assistante" at the Department of Pathology, University of Geneva. In 1997 she joined the World Health Organization Immunology Research and Training center (WHO-IRTC) in the Department of Biochemistry, University of Lausanne where she is now co-director of the advanced WHO/TDR course on Immunology, Vaccinology and Biotechnology applied to Infectious Diseases. In 2007 she was promoted Associate Professor ad personam at the Department of Biochemistry, Faculty of Biology and Medicine of the University of Lausanne. Her main research interest is the role of the innate immune response in the modulation of Leishmania major-specific immune responses, with a focus on the role of neutrophils in this process.

Group members 2006-2007

Cindy Allenbach, PhD student
Floriane Auderset, PhD student
Mélanie Charmoy, PhD student
Saskia Agten, Master student
Florian Desgranges, Master student
Licinia Almeida, Apprentice

Group of Pr F. Tacchini-Cottier

Host defense to intracellular pathogens depends upon both innate and adaptive cell-mediated immune responses. Polymorphonuclear leukocytes (PMN) which belong to the innate immune system can be massively recruited within hours of microbial infection. In addition to their well-established effector function in innate immunity, they have been reported to secrete immunomodulatory cytokines in response to microbial stimuli *in vitro*, and thus may contribute to the development of antigen specific immune responses.
The driving events decisive for $CD4^+$ Th1 or Th2 differentiation have been demonstrated to take place within the first hours to 3 days after infection with *L. major*. PMN are recruited to the site of parasite inoculation already one hour following *L. major* infection and either persist in susceptible mice or decrease to 1-2 % of the cellular infiltrate 3 days after infection in resistant mice.
The role of the newly migrating PMN in the subsequent development of *Leishmania*-reactive T helper immune response within the draining lymph node of resistant or susceptible mice is not yet well characterized and is the focus of our current research.

Interaction of neutrophils with macrophages and transmembrane TNF and their role in the development of *L. major* specific immune response
The early cellular infiltrate at the site of infection with *L. major* shows qualitative and quantitative differences in mice susceptible (BALB/c) or resistant (C57BL/6) to infection with *L. major*. In BALB/c mice, characteristics of an acute inflammatory process such as persistent elevated numbers of neutrophils are sustained whereas in C57BL/6 mice, macrophages are the predominant cell population at the site of parasite inoculation already three days after infection while only a low number of PMNs remains locally.
To understand the mechanisms involved in these differences, we investigated the contribution of PMN apoptosis in this process. Macrophages phagocytose apoptotic PMNs and we showed that they induce neutrophil apoptosis, a process enhanced by *L. major*. We further showed that macrophage-induced PMN apoptosis was dependent on transmembrane TNF, as macrophages isolated from mice genetically deficient in TNF failed to induce neutrophil apoptosis. Using fixed macrophages, macrophages derived from mTNF knock-in mice, and Transwell chambers, we demonstrated that mTNF at the surface of macrophages was necessary to induce PMN apoptosis. We further investigated the role of mTNF during *L. major* infection and showed that the sole presence of transmembrane TNF was sufficient to prevent the development of an inflammatory lesion and efficiently reduce parasite replication. This effect was associated with the rapid clearance of neutrophils at the site of parasite infection. In contrast, mice with total TNF deficiency developed nonhealing inflammatory lesions and neutrophils failed to be cleared from the infection site. Thus, selective targeting of soluble TNF represents a new therapeutic strategy to treat inflammatory diseases and maintain control against intracellular pathogens as shown here for leishmania.

Selected publications WHO-IRTC

- Ronet, C., Voigt, H., Himmelrich, H., Doucey, M.A., Hauyon-La Torre, Y., Revaz-Breton, M., Tacchini-Cottier, F., Bron, C., Louis, J. and Launois, P. (2008) Leishmania major-specific B cells are necessary for Th2 cell development and susceptibility to L. major LV 39 in BALB/c mice. J. Immunol. 180(7), 4825-35.
- Allenbach, C., Launois, P., Mueller, C. and Tacchini-Cottier, F. (2008) An essential role for transmembrane TNF in the resolution of the inflammatory lesion induced by Leishmania major infection. Eur. J. Immunol. 38(3), 720-31.
- McFarlane E., Perez, C., Charmoy, M., Allenbach, C., Carter K.C., Alexander, J. and Tacchini-Cottier, F. (2008) Neutrophils contribute to development of a protective immune response during onset of infection with Leishmania donovani. Infect. Immun. 76(2), 532-41.

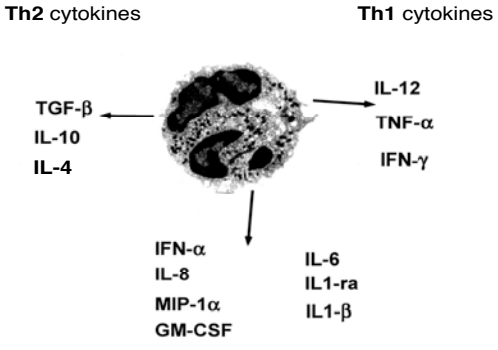


Figure 1: Cytokines production by PMN*. Cytokines involved in CD4⁺Th1 or Th2 differentiation or function have been reported to be secreted by neutrophils.*

Distinct neutrophil phenotypes in mouse strains that are susceptible or resistant to infection with *L. major*

Polymorphonuclear neutrophil granulocytes (PMNs) constitute the first line of defense against infectious agents such as bacteria, fungi and protozoa. They are recruited in large number from the blood to sites of infection. Their best-characterized functions are their role in phagocytosis and killing of invading microorganisms *via* the generation of oxygen intermediates and the release of lytic enzymes stored in their granules. Failure to accomplish some neutrophils functions can lead to severe clinical disorders such as Chronic Granulomatous Disease (LGD) and Leukocyte Adhesion Deficiency (LAD). Neutrophils are short lived, non-dividing cells that until recently were considered as terminally differentiated cells no more capable of protein synthesis. They have since been reported to synthesize in response to microorganism-derived stimuli numerous proteins including chemokines and cytokines.

The diversity of the cytokines produced by PMNs is large, but the magnitude of cytokines production by PMN is generally lower than that of mononuclear cells (10-20 fold less mRNA/ cell). PMN constitute the majority of infiltrating cells in inflamed tissues and thus may nevertheless represent an important source of cytokines in these conditions as shown in Fig.1). PMN, through their secretion of cytokines, may thereby not only play an important role in the initiation and maintenance of inflammatory reactions, but also constitute a link between cells of innate immune system and those of the adaptive immune system.

To investigate the contribution of PMN-secreted cytokines following infection with *L. major*, peritoneally-induced PMNs from BALB/c and C57BL/6 mice were recruited within hours of *L. major* injections and purified by magnetic cell sorter (MACS) using the neutrophil-specific $1A8^{+}$ mAb, which gives a purity of 92-95%. PMNs were cultured *in vitro* and incubated in presence or absence of *L. major* promastigotes. Distinct TLR were induced in neutrophils of resistant *versus* susceptible mice: In response to *L. major* infection, the levelsof TLR2, TLR7, and TLR9 mRNA were significantly higher in C57BL/6 than in BALB/c PMN.C57BL/6 PMN secreted biologically active IL-12p70 and IL-10. In contrast, *L. major*-infected BALB/c PMN transcribed and secreted high levels of IL-12p40 but did not secrete biologically active IL-12p70. Furthermore, IL-12p40 was shown not to associate with IL-23 p19 but formed IL-12p40 homodimers with inhibitory activity. No IL-10 was secreted by BALB/c PMN. Thus, following infection with *L. major*, in contribute to impaired, early IL-12 signaling. These distinct PMN phenotypes may thus influence the development of *L. major*-specific immune response. We are currently pursuing these studies to better understand the contribution of neutrophils to the local microenvironment shaping the immune response to *L. major* parasite, focusing on the crosstalk between neutrophils and dendritic cells.

Both groups (P. Launois and F. Tacchini-Cottier)

Analysis of human immune responses to different Leishmania species

Human cutaneous leishmaniasis is caused by many species of *Leishmania* having different geographic distribution and spectra of disease. Cutaneous manifestations of leishmaniasis can result from infection with either viscerotropic or dermatotrophic species. In the Old World *L. major*, *L. tropica* and visceralizing L. *infantum*, and *L donovani* complex member

Selected publications WHO-IRTC

- Charmoy, M., Megnekou, R., Allenbach, C., Zweifel, C., Perez, C., Monnat, K., Breton, M., Ronet, C., Launois, P. and Tacchini-Cottier, F. (2007) Leishmania major induces distinct neutrophil phenotypes in mice that are resistant or susceptible to infection. J. Leukoc. Biol. 82, 288-299.
- Kariminia, A., Bourreau, E., Ronet, C., Couppie, P., Sainte-Marie, D., Tacchini-Cottier, F. and Launois, P. (2007) Selective expression of the V beta 14 T cell receptor on Leishmania guyanensis--specific CD8+ T cells during human infection. J. Infect. Dis. 195, 739-747.
- Olleros, M.L., Vesin, D., Martinez-Soria, E., Allenbach, C., Tacchini-Cottier, F., Pache, J.C., Marchal, G., Rahman, J., Fernandez, C., Izui, S. and Garcia, I. (2007) Interleukin-12p40 overexpression promotes interleukin-12p70 and interleukin-23 formation but does not affect bacille Calmette-Guerin and Mycobacterium tuberculosis clearance. Immunology 122, 350-361.
- Bourreau, E., Ronet, C., Couppie, P., Sainte-Marie, D., Tacchini-Cottier, F. and Launois, P. (2007) IL-10 producing CD8+ T cells in human infection with Leishmania guyanensis. Microbes Infect. / Institut Pasteur 9, 1034-1041.
- Allenbach,C., Zufferey, C., Perez, C., Launois, P., Mueller, C. and Tacchini-Cottier, F. (2006) Macophages induce neutrophil apoptosis through mTNF, a process amplified by *L. major*. J. Immunol. 176 (11), 6656-6664.
- Gumy, A., Aseffa, A., Rachinel, N., Breton, M., Otten, L.A., Tacchini-Cottier, F., Rocken, M., Doyen, N., Acha-Orbea, H., Locksley, R., MacDonald, R., Launois, P. and Louis, J.A. (2006) Lack reactive CD4+ T cells require autocrine IL-2 to mediate susceptibility to L. major. Eur. J. Immunol. 36, 1-9.

(*L. archibaldi*) on the other hand can also cause cutaneous manifestations. Dermal lesions at the inoculation site of *L. infantum* or *L. archibaldi* resemble lesions caused by dermatotrophic species whereas *L. donovani* is unique in producing disseminated non-ulcerating dermal lesions in some individuals following visceral leishmaniasis, or PKDL, post Kala Azar dermal leishmaniasis. In the New World, *L. mexicana*, *L. amazonensis*, *L. braziliensis*, *L. panamensis*, *L guyanensis*, and *L. peruviana* are the most frequent causes of dermal leishmaniasis and *L. chagasi*, which causes visceral leishmaniasis in the Latin America also presents cutaneous manifestations similar to lesions caused by the previously mentioned species. The immunopathogenesis of the different forms of dermal leishmaniasis is poorly understood and the immunologic mechanisms of resistance to the diverse species involved are undefined. To investigate if the mechanisms of resistance and susceptibility to infection with *Leishmania* deciphered in mice have their counterpart in humans, we are developing work in humans in collaborations with different institutions from endemic countries from both Old and new World (Colombia and French Guiana for the New World; Sudan and Tunisia for the Old World).

Cytokines are increasingly recognized as important components of the cellular immune responses to intra cellular pathogens. Thus, we analyzed the production of TGF-β, IL-10 and IFN-γ by PBMC of unexposed naïve subjects and LCL patients after stimulation with live L.g. We demonstrated that IFN-g is produced in controls and LCL patients, IL-10 only in LCL patients and TGF-β only in naïve subjects. Furthermore, in naive subjects, neutralization of TGF-β induced IL-10 production. IL-10 produced in naïve subjects when TGF-β is neutralized or in LCL patients did not modify the IFN-γ production but inhibit Reactive Nitrogen Species production. Analysis of the phenotype of IL-10 producing cells in naive subjects when TGF-β is neutralized clearly showed that they are memory $CD45RA^{-}$ $CD8^{+}$ T cells. In LCL patients, IL-10 producing cells are both $CD45RA^{-}$ CD4 and $CD8^{+}$ T cells.

In collaboration with the Institute Pasteur in French Guiana, we have also analyzed the Vβ TCR repertoire expression by RT-PCR ELISA in different clinical presentation of human infection with *Leishmania guyanenis* (L.g.). We demonstrated that L.g. stimulated CD8+ T cells produced IFN-γ and expressed preferentially the Vβ14 TCR gene family. In addition, these cells expressed the CLA and CCR4 surface molecules suggesting that they could migrate to the skin. Results obtained with lesions of LCL patients showed that the TCR Vβ14 expression was increased in most of the lesions (63.5%), whereas only a small number of Vβ gene families was increased (Vβ1, 6, 9, 14 and 24). The presence of Vβ14 T cells in tissues confirms the migration of these cells to the lesion site. Thus, we propose the following sequence of events during infection with Lg. After the first exposure to L.g., CD8+ T cells expressing preferentially the Vβ14 TCR and secreting IFN-γ develop and circulate in the periphery. During the infection, they migrate to the skin at the site of parasite infection.

The role of these IFN-γ an IL-10 producing CD8+ T cells in the resistance to infection remains to be firmly evaluated.

Pascal Schneider,
Assistant Professor

Pascal Schneider studied biochemistry and obtained a PhD in 1992 at the University of Lausanne. He performed a post-doc with MAJ Ferguson at the University of Dundee, Scotland, and then joined the research group of J. Tschopp at the Department of Biochemistry, University of Lausanne, where he has been appointed assistant professor in 2002 and senior lecturer and researcher in 2006. Ligands and receptors of the TNF family are his major research interest.

Group members 2006-2007

Aubry Tardivel, Research associate
Karine Ingold, Technician
Laure Willen, Technician
Claudia Bossen, PhD student
Sandrine Ballenegger, Diploma student

Functional characterization of TNF family ligands

TNF family ligands are trimeric, type II transmembrane proteins that can either function as membrane-bound proteins or as soluble cytokines after proteolytic processing. Although they are predominantly active in the development, homeostasis and function of the immune system, they are also involved in processes as diverse as regulation of energy metabolism, control of bone turnover, development of the mammary gland and morphogenesis of skin derivatives such as hair, teeth and sweat glands. Primary defects in TNF family ligands or their receptors are directly associated to several inherited diseases, whereas deregulated expression of some members is implicated in the onset and/or maintenance of autoimmune conditions or malignancies. The TNF/TNFR family thus provides a number of interesting therapeutic targets.

BAFF and APRIL exist in several forms with differential impact on the biology of B cells

Production of antibodies is important for immunity. Indeed, a basal level of antibody is sufficient to confer protection against a wide variety of common bacteria, as shown by the success of passive antibody transfer in patients with antibody deficiencies. In addition to these "natural" antibodies usually directed against common antigenic patterns of microorganisms, the immune system can produce highly specific antibodies targeting a specific antigen such as a toxin. Such a response to a novel antigen develops within a few days to a few weeks, depending on the quality of the antibody. Antibody-secreting cells are derived from B-lymphocytes, which undergo a number of developmental and maturation steps before they reach the fully differentiated stage of plasma cells that are dedicated to antibody production.
The TNF family ligands BAFF and APRIL regulate various aspects of B cell biology. BAFF binds to the receptors TACI, BCMA and BAFF-R, whereas APRIL binds to TACI and BCMA only. We have recently shown that APRIL also interacts with negatively charged side chains of proteoglycans (Figure 1).

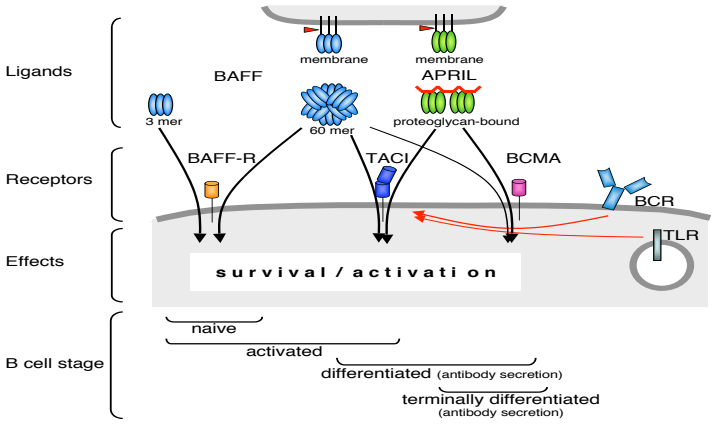


***Figure 1**: BAFF and APRIL promote survival of B cells at different stages of activation/ differentiation through different receptors. Naïve B cells express BAFF-R through which they receive survival signals. Soluble BAFF 3-mer, but not APRIL, can activate this receptor. The signals provided by BAFF 3-mer through BAFF-R probably regulates the size of the B cell pool. TACI is induced upon B cell activation, for example through triggering of the B cell receptor (BCR) or Toll-like receptors (TLR). BCMA is active in antibody-secreting cells. TACI can only signals in response to oligomeric forms of BAFF and APRIL (membrane-bound, 60-mer, proteoglycan-bound), and is probably crucial for the generation of at least some kind of plasma cells, such as those elicited in response to T-independent type II antigens.*

Using genetically-deficient mice and various purified forms of BAFF and APRIL, we have studied the functional relevance of BAFF and APRIL signaling in B cell at various stages of differentiation. Results indicated that BAFF-R is the prime receptor used for the survival of naïve B

Selected publications

- Bossen, C., Cachero, T., Tardivel, A., Ingold, K., Willen, L., Dobles, M., Scott, M.L., Maquelin, A., Belnoue, E., Siegrist, C.A., Chevrier, S., Acha-Orbea, H., Leung, H., Mackay, F., Tschopp, J. and Schneider, P. (2008) TACI, unlike BAFF-R, is solely activated by oligomeric BAFF and APRIL to support survival of activated B cells and plasmablasts. Blood 111, 2755-2764.
- Casal, M.R., Lewis, J.R., Mauldin, E.A., Tardivel, A., Ingold, K., Favre, M., Paradies, F., Demotz, S., Gaide, O. and Schneider, P. (2007) Significant correction of disease after post-natal administration of recombinant EDA in canine X-linked ectodermal dysplasia . Am. J. Hum. Genetics 81, 1050-1056.
- Schwaller, J., Schneider, P., Mhawech-Fauceglia, P., McKee, T., Myit, S., Matthes, T., Tschopp, J., Donzé, O., Le Gal, F.A. and Huard, B. (2007) Neutrophil-derived APRIL concentrated in tumor lesions by proteoglycans correlates with human B cell lymphoma aggressiveness. Blood 109, 331-338.
- Cachero, T.G., Schwartz, I.M., Qian, F., Day, E.S., Bossen, C., Ingold, K., Tardivel, A., Krushinskie, D., Eldredge, J., Silvian, L., Lugovskoy, A., Farrington, G.K., Strauch, K., Schneider, P. and Whitty, A. (2006) Formation of Virus-like Clusters Is an Intrinsic Property of the Tumor Necrosis Factor Family Member BAFF (B Cell Activating Factor). Biochemistry 45, 2006-2013.
- Bossen, C., Ingold, K., Tardivel, A., Bodmer, J.L., Gaide, O., Hertig, S., Ambrose, C., Tschopp, J. and Schneider, P. (2006) Interaction of TNF and TNF receptor family members in the mouse and human. J. Biol. Chem. 281, 13964-13971.
- Mou, C., Jackson, B., Schneider, P., Overbeek, P., Headon, D.J. (2006) Generation of primary hair follicle pattern. PNAS. 103, 9075-9080.
- Ingold, K., Zumsteg, A., Tardivel, A., Huard, B., Steiner, Q.-G., Cachero, T., Qian, F., Gorelik, L., Kalled, S.L., Acha-Orbea, H., Rennert, P.D., Tschopp, J. and Schneider, P. (2005) Identification of proteoglycans as APRIL-specific binding partners. J. Exp. Med. 209, 1375-1383.

splenocytes, with no or very little contribution of the other receptors. As a consequence of the specificity of BAFF-R for BAFF, naïve B cells did not respond to APRIL. After stimulation of the B cell receptor, an activation signal known to upregulate TACI, B cells could receive co-stimulatory signals (survival and activation) through both BAFF-R and TACI, and therefore responded to both BAFF and APRIL. Plasmablasts and plasma cells elicited in response to viral infection or immunization with various antigens used TACI and BCMA instead of BAFF-R to respond to BAFF and APRIL. Thus, as B cell differentiate from the naïve stage to the fully differentiated antibody-secreting cell stage, they switch their BAFF and APRIL receptors from BAFF-R to TACI and from TACI to BCMA. This results in progressive acquisition of APRIL-responsiveness and a reduced responsiveness to BAFF.

The form of the ligand that is used to trigger the different receptors plays an important role. BAFF occurs in both membrane-bound and soluble forms. Soluble BAFF can be found as a 3-mer or as a highly ordered structure containing twenty 3-mer (60-mer). Soluble APRIL 3-mer can also potentially multimerize through its interaction with proteoglycans. In any case, the oligomerization status of the ligand has a dramatic impact on its ability induce a signal through a given receptor. Thus, although BAFF-R responds to all kinds of BAFF, TACI can only signal in response to membrane-bound BAFF, BAFF 60-mer or APRIL oligomers, but not to BAFF 3-mer or APRIL-3mer. The requirements for BCMA appear to be similar to those of TACI (Figure 1). These finding suggest a model in which BAFF 3-mer is primarily responsible for the maintenance of the naïve B cells pool. Upon antigen-encounter, activated B cells and newly formed plasmablasts receive positive signals from oligomeric forms of BAFF, such as those provided by membrane-bound BAFF upon cell-cell contact. Finally, plasma cells may find a favorable environment in APRIL-rich niches in which APRIL would be concentrated on proteoglycan of the extracellular matrix or of the plasma cells themselves. This model suggest an important role for the APRIL-TACI axis in the proper generation and/or maintenance of antibody-secreting cells, and is in line with the observation that mutations in TACI (some of which prevent binding to APRIL) are associated with common variable immunodeficiency (CVID), a condition characterized by low circulating antibody levels and recurrent infections.

EDA controls the development of ectodermal appendages, and can be used for protein replacement therapy in animal models of EDA-deficiency.

Whereas most TNF family ligands are intimately involved in the function of the immune system, EDA is a notable exception in that it is required for the development of ectodermal appendages such as hair, teeth and sweat glands. Deficiencies in both EDA or its receptor EDAR lead to a condition known as hypohidrotic ectodermal dysplasia (HED) that can be life-threatening in young affected individuals during episodes of fever. Indeed, the severe deficiency of sweat gland prevents the regulation of hyperthermia by sweating. Other symptoms that impinge on the quality of life include the absence or malformation of teeth, a dry-eye phenotype and susceptibility to recurrent infections of the respiratory tract (probably due to the abnormal function of mucus-secreting glands in the bronchi and trachea that impairs mechanical excretion of air-born particles).

The mechanism of action of EDA has been studied mainly in the development of hair follicles. EDA must be released in a soluble form to engage its receptor EDAR and to activate the NF-κB transcription factor. This induces expression of proteins, in particular CTGF/CCN2, that may act to remove inhibitory signals of hair follicle formation, and therefore allow for the initiation of a placode, the hair follicle primordia (Figure 2).

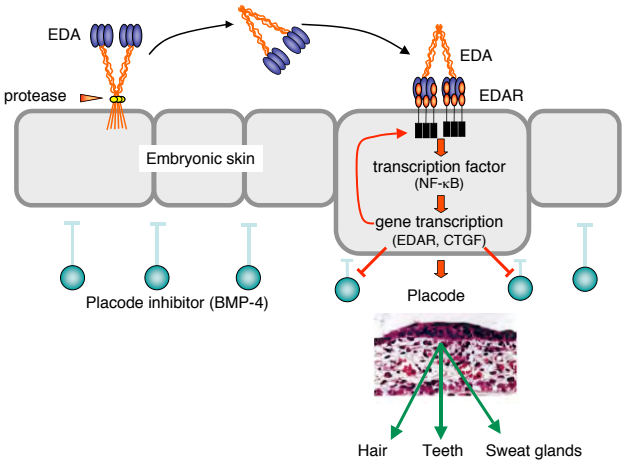


***Figure 2**: Involvement of the TNF family ligand EDA in the development of skin appendages. Membrane-bound EDA must be released as a soluble protein in order to activate its receptor, EDAR. At the site of EDAR activation, a NF-kB-dependent pathwayinduces neutralization of placode inhibitors, thus opening the way to the development of skin-derived structures such as hair, teeth or sweat glands. Hair and teeth that form despite the absence of EDA are often morphologically abnormal, suggesting that EDA is required for development and morphology.*

Our earlier observations indicated that recombinant EDA could substitute at least partially for the missing endogenous EDA in EDA-deficient mice. In collaboration with Dr Magi Casal (University of Philadelphia), we have treated newborn EDA-deficient dogs with recombinant EDA. Dogs are indeed much more similar to human than mice are with respect to many criteria, for example the presence of both deciduous and permanent teeth, whereas mice have only permanent teeth. The results indicate that a short course, post-natal treatment with recombinant EDA improved a number of the clinical symptoms of the disease. Formation of functional sweat glands was observed in treated animals, lacrymation was normalized (so that daily treatment with eye drops became unnecessary), the function of mucus secreting glands in the airways was improved to a level that completely prevented the occurrence of the airways infections frequently observed in EDA-deficient dogs, and the secondary dentition was corrected in a spectacular manner (Figure 3). These data provide a strong proof-of-concept that administration of recombinant EDA may provide long-term benefit to human babies afflicted with HED.

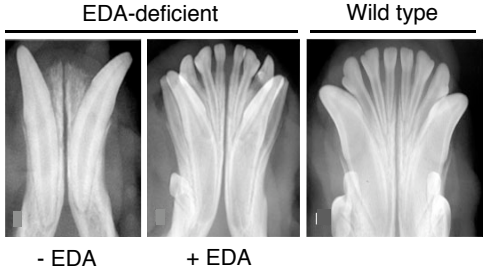
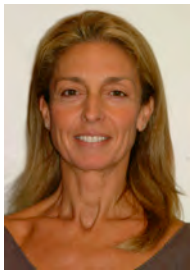


***Figure 3**: A recombinant form of EDA rescues the permanent dentition in a dog model of EDA-deficiency.*

Catherine Servis, Project leader



Catherine Servis received her PhD from the University of Heriot-Watt in Edinburgh on studies involving the localization, identification and synthesis of the immunodominant determinants of Lactate Dehydrogenase-B4 and IgG2a myeloma protein.After a postdoctoral training at the Basel Institute for Immunology (Hoffmann-La-Roche) she continued her training in protein chemistry at the Friedriech-Miescher Institute (Ciba Geigy–Novartis). In 1990, she joined the Institute of Organic Chemistry of the University of Lausanne where she was involved in the development of strategies for the solid phase synthesis of molecules for biological applications. She joined the Institute of Biochemistry in 1992 where she is heading the Protein and Peptide Chemistry Facility. She is head of the Clinical Tumor Proteome Analysis Facility since May 2005.

Interests: Development of clinical proteomic strategies for the identification and quantification of biomarkers in biofluids and tissue from cancer patients. Development of synthetic tools for biological applications.

Group members 2006-2007

Florela Penea, Technician
Luis Rodrigues, Technician
Vincent Studer, Trainee

Development of proteomic strategies for biomarker discovery and synthetic tools for biological applications

Clinical proteomics

Cancer-specific markers in plasma which may be useful for cancer detection and monitoring include proteins released in smaller amounts from cancer tissues (tissue-specific proteins) or as a result of structural and functional changes in the microenvironment surrounding cancer cells. They may also include mediator or effector molecules of the native or acquired immune response to cancer cells (e.g. auto antibodies to cancer-specific proteins). For these reasons, most cancer markers currently in clinical use are low-abundance proteins with concentrations in the nanogram per milliliter range in plasma.
Proteomics technology promises to be a valuable way to identify proteins and fragments thereof present in the tumor environment that may be used to define new molecules for diagnostic, prognostic and therapeutic purposes. Recently, as many as 1261 proteins believed to be differentially expressed in human cancer patients relative to healthy individuals, have been proposed as candidate plasma biomarkers that could be useful in early cancer detection and monitoring, given sufficiently sensitive and specific tests are available for their detection. In the last decade, different proteomics platforms based on Mass Spectrometry (MS), have been developed and used for the identification of cancer-associated proteins and peptides in tissue and serum.

Activities

1. The Protein and Peptide Chemistry Facility
(PPCF**,** www.unil.ch/ib/page9229.html) provides synthetic tools for various biological applications and a variety of analytical and technical services using mass spectrometry to the Lausanne scientific community.
The Facility is also involved in research and teaching on different aspects of protein and peptide chemistry.

2. The Clinical Tumor Protein Analysis Facility
(CTPAF, prior within the NCCR program, www.nccr-oncology.ch/scripts/page9249.html) is now included within the Molecular Biomarker Facility (MBF) of the nascent Lausanne Cancer Center (LCC). The goal of this platform is to develop and optimize strategies for the identification and quantification of molecular markers in biofluids and tissue of cancer patients.

Selected publications

- Villard, V., Agak, G.W., Frank, G., Jafarshad, A., Servis, C., Nebie, I., Sirima, S.B., Felger, I., Arevalo-Herrera, M., Herrera, S., Heitz, F., Backer, V., Druilhe, P., Kajava, A.V. and Corradin, G. (2007) Rapid identification of malaria vaccine candidates based on alpha-helical coiled coil protein motif. PLoS ONE. 2(7), e645.
- Chapatte, L., Ayyoub, M., Morel, S., Peitrequin, A.L., Levy, N., Servis, C., Van den Eynde, B.J., Valmori, D. and Levy, F. (2006) Processing of Tumor-Associated Antigen by the Proteasomes of Dendritic Cells Controls In vivo T-Cell Responses. Cancer Res. 66(10), 5461-8.

Research projects

Monitoring proteins relevant to tumor angiogenesis and tumor progression by proteomic analysis of biofluids and tissue from cancer patients

In collaboration with the groups of C. Ruegg (CePO, LCC, UNIL) M. Hegi (Lab. of Tumor Biology and Genetics, CHUV) and R. Stupp (CePO, LCC, CHUV, UNIL)

We are planning to follow two approaches:

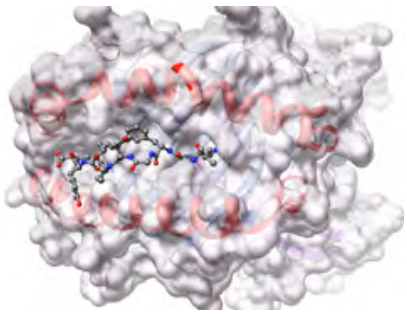
i) Targeted analysis of molecules in biofluids and tissues. In a hypothesis-driven approach we will accurately identify and quantify selected proteins in CSF and serum from patients with multiple glioblastoma or other solid tumors (e.g. colon, breast, head and neck cancers). We will detect molecules that have been previously identified by a single biomarker approach (e.g. VEGF-A, MMP-9) and molecules that have been identified as potential candidates of angiogenesis or tumor progression in gene expression studies (e.g. Cyr61/CCN1; GDF3, GDF-15, EPSTI-1). We will employ a quantitative proteomics approach developed by Aebersold which is based on the selection and chemical synthesis of isotopically labeled reference peptides corresponding to peptides generated from the native protein by tryptic digestion. Since the proteins of interest are glycoproteins, we will use the glycoprotein enrichment method which is developed by the same group and further analysis by LC-ESI MS in SRM/MRM mode in the presence of the reference peptide. This approach has been proven suitable for detecting a panel of candidate biomarkers in clinical samples.

ii) Global protein profiling in biofluids and cancer tissues. Once this first approach has been successfully established, we plan to analyze tumor tissue and biofluids for global protein expression profiles (signatures) specific to the tumor, its propensity to progress (patient survival) and (possibly) its response to therapy. We will study only patients for which clinical follow up and/or patient outcome is known. The proposed strategy involves fractionation of the material to ensure characterization of the different types of components (e.g. small vs large proteins). After immunodepletion of the high abundant proteins, samples will be reduced, alkylated and digested by trypsin and generated peptides separated by 2-D LC and analyzed by MALDI-TOF/TOF. Quantitative differences in the proteins present in tumor tissue or serum/CSF samples will be initially determined through comparison of the peak intensities of their constituent peptides/proteins. Later, when specific peaks have been characterized as interesting, we will quantitate the corresponding proteins by the use of isotopically labeled reference peptides (see above).

Mass Spectrometry-based strategy to measure alteration of glycosylation

In collaboration with Bruno Domon, ETH – IMSB.

The aim of this project is to detect the alteration of the glycosyltion pattern in the serum of cancer patients by differential analyses using stable isotope labelling. The method developed includes cleavage of N-linked glycans from glycoproteins in serum using PNGase F, and derivatization of the glycans with 2-aminopyridine (or an isotopically labeled variant). The reductively aminated samples are mixed, chemically desialylated and analyzed on a quadrupole time-of-flight system coupled to a micro-HPLC system and a microfluidic device. The different glycoforms are assigned based on their molecular mass, and their structure is confirmed by their



Peptide-MHC

MS/MS patterns. The relative ratio of the signal of one glycoform reflects the abundance of the glycan in the two samples.
We will apply this method to the relative quantification of glycans in the serum of patients with colon, breast cancer and controls. In addition to the analysis of the glycans from the whole serum, glycan patterns will also be analyzed after the depletion of the major high abundant proteins in serum. This will provide information about N-glycans from low abundant proteins which may include membrane surface proteins that play an important role in cancer development.

In vitro degradation assay as a means to analyze the production of peptide tumor antigens
In collaboration with Frédéric Lévy (Ludwig Institute for Cancer Research, Lausanne Branch)
The proteasome plays a crucial role in the proteolytic processing of antigens presented to T cells in the context of MHC class I molecules. However, the rules governing the specificity of cleavage sites are still largely unknown. We developed a new technique for the identification of degradation products. Using purified proteasome and MALDI mass spectrometry for identification, it has been possible to rapidly and unambiguously identify degradation products corresponding to antigenic peptides. We can analyze the production of a limited number of candidate peptide tumor antigens, which has been identified on the basis of their restricted expression pattern and binding motif for particular MHC class I molecules. We can now analyze several precursors of peptide tumor antigens and include this test in the characterization of each newly identified potential peptide tumor antigen. Introduction of this new technique may prove important as several potential peptide tumor antigens have been shown to be destroyed by the proteasome.

The identification of new peptide tumor antigens derived from alternatively spliced variants expressed specifically in neoplastic cells (NCCR)
Within this project, our main focus is the analysis (separation and identification) and quantitation of potential HLA class I-restricted ligands generated by proteasomal degradation of precursor peptides encoded by alternatively expressed exons. The proteolytic activities of other peptidases involved in the antigen processing pathway will also be studied and analyzed.

Margot Thome, Assistant Professor

Margot Thome studied Biochemistry at the University of Tübingen, Germany, and at the University of Arizona, USA. In 1993 she joined the laboratory of Oreste Acuto at the Pasteur Institute, Paris, where she worked on the role of tyrosine kinases in T-cell activation, and received her PhD from the University of Paris in 1995. Since 1996 she has worked in the Department of Biochemistry at the University of Lausanne. As a postdoctoral fellow she studied viral and cellular regulators of apoptosis in the laboratory of Jürg Tschopp. Since 2004, she holds an SNF Assistant Professorship. Her present research focuses on signaling pathways that control lymphocyte activation and survival.

Molecular mechanisms of lymphocyte activation and survival

Lymphocytes play a crucial role in the defense against pathogens and tumor cells. One focus of our research is to understand the molecular mechanisms that control the activation of T-lymphocytes, initiated upon triggering of the T-cell antigen receptor by MHC-bound antigen. This leads to the initiation of multiple signaling pathways that regulate changes in cell shape and gene expression that are critical for efficient T-cell activation, proliferation and survival. Another focus of our research is to understand the molecular mechanisms underlying aberrant lymphocyte proliferation and survival that occurs in certain lymphoid tumors (lymphomas).
By uncovering new molecular players and enzymatic activities relevant to these pathways, we aim at identifying possible targets for therapeutic immuno-modulation or treatment of lymphomas.

T-cell receptor induced changes in cell shape and gene expression

The initiation of the adaptive immune response depends on the recognition of pathogenic substances or tumor-specific molecules (microbial or tumor antigens) by cell surface receptors on lymphocytes. Upon antigen recognition, lymphocytes undergo dramatic changes in cell shape and gene expression, which contribute to the activation, clonal proliferation and survival of the stimulated lymphocytes, which become effector cells capable of eliminating infected or tumor cells.
We are studying a complex of proteins, comprising CARMA1, BCL-10 and MALT1 that play key roles in the initiation of the adaptive immune response (Fig. 1). An important role for these proteins, which we and others have identified over the last few years, is the activation of the transcription factor NF-κB, which in turn controls the expression of genes that are essential for lymphocyte proliferation and survival (Gaide et al., 2002; Thome, 2004). More recently, we have identified another key role for this complex of proteins in the control of cellular adhesion processes that are important for the recognition of target cells by lymphocytes (Rebeaud, Hailfinger et al., 2008). Finally, we have described an essential role for BCL-10, independently of its association with CARMA1 or MALT1, in the control of T-cell- and Fc-receptor mediated actin polymerization, and could show that this is important for the recognition of antigen-presenting target cells by T-lymphocytes, but also for the Fc-receptor-mediated phagocytic uptake of antibody-covered pathogens (Rueda et al., 2007).

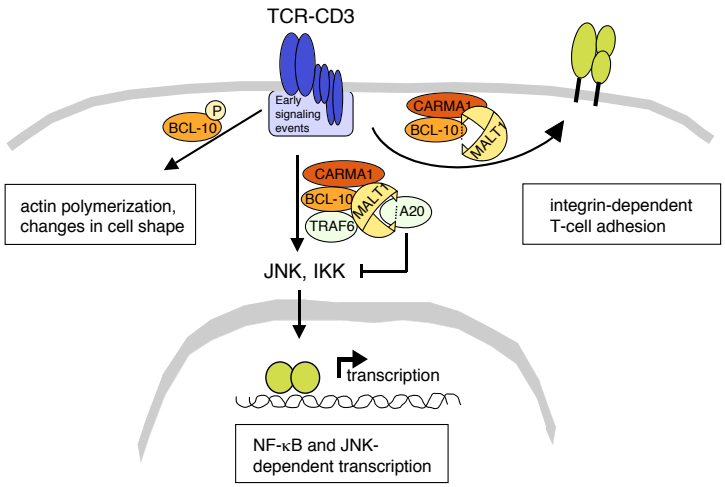


***Figure 1*: CARMA1, BCL-10 and MALT1 have multiple functions in T-cell activation.** *We could show that T-cell receptor (TCR)-induced phosphorylation of BCL-10 is critical for the rapid induction of actin polymerization and actin-dependent changes in cell shape. TCR-induced formation of a CARMA1-BCL-10-MALT1 (CBM) complex is essential for activation of the c-jun kinase (JNK) and NF-κB transcriptional pathways that regulate transcription of genes that control T-cell activation, proliferation and survival. It is thought that in this context, MALT1 contributes to NF-κB activation by recruitment of the ubiquitin ligase TRAF6 and by proteolytic cleavage of proteins with inhibitory function, such as A20. Recently, we could show that CBM complex formation results in the proteolytic cleavage of BCL-10, which is not required for NF-κB activation but essential for integrin-dependent T-cell adhesion.*

Group members 2006-2007

Montserrat Guzzardi, Technician
Licinia Almeida, Apprentice
Anita Posevitz-Fejfar, Postdoctoral fellow
Daniel Rueda, Postdoctoral fellow
Stephan Hailfinger, PhD student
Roger Moser, PhD student
Fabien Rebeaud, PhD student
Myriam Tapernoux, PhD student

Selected publications

- Rebeaud, F., Hailfinger, S., Posevitz-Fejfar, A., Tapernoux, M., Moser, R., Rueda, D., Gaide, O., Guzzardi, M., Iancu, E.M., Rufer, N., Fasel, N. and Thome, M. (2008) The proteolytic activity of MALT1 is key to T-cell activation. Nat. Immunol. 9, 272-281.
- Rueda, D., Gaide, O., Ho, L., Lewkowicz, E., Niedergang, F., Hailfinger, S., Rebeaud, F., Guzzardi, M., Conne, B., Thelen, M., Delon, J., Ferch, U., Ruland, J., Mak, T., Schwaller, J. and Thome, M. (2007) Bcl10 controls T-cell receptor- and FcgR-induced a54tin polymerization. J. Immunol. 178, 4373–4384.
- Thurau, M., Everett, H., Tapernoux, M., Tschopp, J. and Thome, M. (2006) The TRAF3 binding site of human molluscipox virus FLIP molecule MC159 is critical for its capacity to inhibit Fas-induced apoptosis. Cell Death Differ. 13, 1577-1585.
- Teixeiro, M., Daniels, M.A., Hausmann, B., Schrum, A.G., Naeher, D., Luescher, I., Thome, M., Bragado, R. and Palmer, E. (2004) T Cell Division and Death Are Segregated by Mutation of TCR Chain Constant Domains. Immunity 21, 515-526.
- Egawa, T., Albrecht, B., Favier, B., Sunshine, M.-J., Mirchandani, K., O'Brien, W., Thome, M. and Littman, D.R. (2003) Requirement for CARMA1 in antigen receptor-induced NF-kB activation and lymphocyte proliferation. Curr. Biol. 13, 1252-1258.
- Gaide, O., Favier, B., Legler, D., Bonnet, D., Bron, C., Valitutti, S., Tschopp, J. and Thome, M. (2002) Carma1 is a critical lipid raft-associated regulator of T-cell receptor-induced NF-kB activation. Nature Immunol. 3, 836-843.

The proteolytic activity of MALT1 is key to T-cell activation

The transcription factor NF-κB plays a key role in the expression of genes that are essential for lymphocyte activation and the generation of the immune response. In resting lymphocytes, NF-κB family members are present in the cytoplasm in an inactive form, bound to inhibitory κ-B (IκB) proteins. Triggering of the T-cell antigen receptor leads to activation of the IκB kinase (IKK) complex that induces phosphorylation and subsequent degradation of IκB proteins. This allows NF-κB to translocate into the nucleus and to initiate the transcription of genes that control lymphocyte proliferation and survival.

A major issue in the field of T-cell activation has been to understand the molecular mechanisms linking T-cell receptor engagement to the activation of the IKK complex. One of the earliest events following T-cell receptor (TCR) engagement is the activation of tyrosine kinases and the tyrosine phosphorylation of a restricted set of substrates. These in turn control the activation of Ser/Thr kinases of the protein kinase C family. PKCtheta and PKCbeta are T- and B-cell specific PKC family members essential for antigen receptor-induced NF-κB activation, by phosphorylation of CARMA1. We and others have previously identified CARMA1 as an essential signaling component in the antigen-recptor-induced NF-κB pathway, and shown that a caspase-recruitment domain (CARD)-mediated interaction between CARMA1 and BCL-10 is critical for signal transmission to the IKK complex (Gaide et al., 2002; Thome, 2004).

More recently, we could show that the BCL-10- and CARMA1-binding protein MALT1 contributes in a previously unknown manner to NF-κB activation. In particular, we could show that the proteolytic activity of the C-terminal caspase-like domain of MALT1 is critical for optimal NF-κB activation in T cells (Rebeaud, Hailfinger et al., 2008). MALT1 had been identified several years ago as a protein that shares homology with proteases of the caspase family, but despite intensive efforts it had remained enigmatic whether MALT1 has proteolytic activity and whether this might contribute to NF-κB activation. Through the identification of a BCL-10 cleavage product that is present exclusively in activated T- and B-cells (see below), we could recently demonstrate that MALT1 has an unexpected, Arginine-directed proteolytic activity that is transiently induced upon T-cell stimulation and cleaves the C-terminal part of BCL-10 (Fig. 2a). The development of a peptide-based inhibitor of MALT1 (in collaboration with the group of Prof. Nicolas Fasel), has allowed us to show that the proteolytic activity of MALT1 (but not the cleavage of BCL-10) is required for optimal NF-κB activation and cytokine production in human T cells (Fig. 2b). Since over-expression and/or constitutive activity of MALT1 has been associated with lymphomas of the mucosa-associated tissue (MALT lymphomas) and certain forms of diffuse large B-cell lymphomas (ABC-type DLBCL), these findings identify the proteolytic activity of MALT1 as a highly interesting target for the development of immuno-modulatory and anti-lymphoma drugs. Further studies are now targeted at the identification of the NF-κB-relevant MALT1 substrate(s).

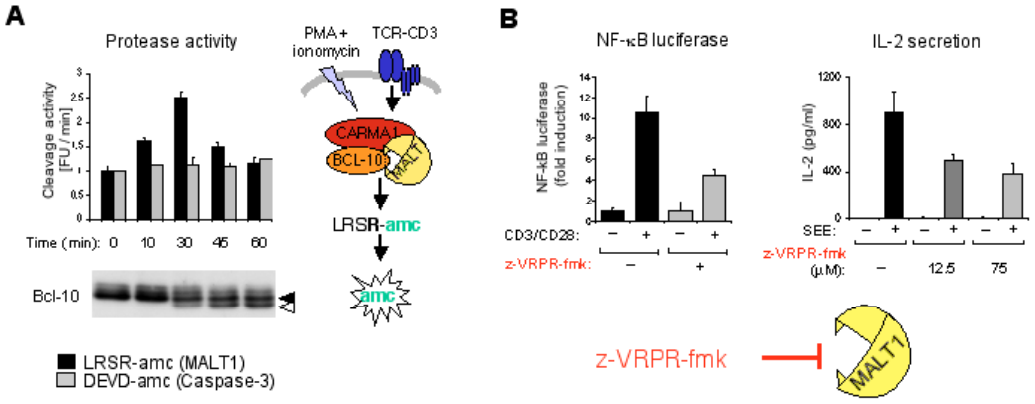


***Figure 2**: **T-cell activation induces MALT1 activation and cleavage of BCL-10. (A)** Analysis of MALT1 activity and BCL-10 cleavage in T cells activated for the indicated times with PMA and ionomycin to mimic natural T-cell activation. T-cell activation leads to a transient increase in MALT1 activation (but not caspase-3 activation) that peaks at 30 min after stimulation. MALT1 activation correlates with the generation of a BCL-10 cleavage product that is stable for many hours (data not shown). **(B)** Treatment of human Jurkat T cells with a cell permeable MALT1 inhibitor, z-VRPR-fmk, leads to impaired NF-κB activation upon stimulation with agonistic anti-CD3 and anti-CD28 antibodies and to reduced secretion of the cytokine IL-2 upon stimulation with superantigen (SEE)-presenting Raji cells (Rebeaud, Hailfinger et al., 2008).*

BCL-10 controls TCR-induced actin polymerization and adhesion

BCL-10 plays a key role in antigen receptor-induced NF-kB activation, but it also has NF-kB independent functions that we have recently started to characterize (reviewed in Thome, 2004; Thome & Weil, 2007). The starting point for these studies was the observation that, in activated T cells, Bcl10 undergoes characteristic post-translational modifications (Fig. 3a) (Rueda et al., 2007; Rebeaud, Hailfinger et al., 2008). We could show that BCL-10 is rapidly phosphorylated on at least two residues, and have identified Serine 138 as critical for BCL-10 phosphorylation. The study of a non-phosphorylatable Serine138-to-Alanine mutant of BCL-10 has revealed that BCL-10 phosphorylation on this site is not required for NF-kB activation, but rather plays a key role in TCR-induced cell shape changes that are critical for the recognition of the antigen-presenting cells by T cells (Rueda et al., 2007). In collaboration with the group of Dr. Florence Niedergang (Institut Cochin, Paris), we could further show that BCL-10-dependent actin polymerization plays an essential role in the Fc-receptor-induced phagocytosis of antibody-coated particles by macrophages, which is a key event in the elimination of pathogens by the immune system.

More recently, we have identified another posttranslational modification of BCL-10, which is due to the proteolytic removal of five amino acids from the C-terminus of BCL-10 by MALT1 (Fig. 3a) (Rebeaud, Hailfinger et al., 2008). The MALT1-dependent BCL-10 cleavage was generated with kinetics corresponding to MALT1 activation (see Fig. 2a) but remained stable for many hours after activation. Treatment of T cells with a MALT1-inhibitor preventing BCL-10 cleavage or reconstitution of BCL-10-deficient cells with a non-cleavable mutant of BCL-10 led to a clear reduction of TCR-induced adhesion of the T cells to fibronectin, thus demonstrating an impaired capacity of the T-cells to undergo integrin-mediated adhesion. In contrast, BCL-10 cleavage was not required for NF-kB activation.

Selected reviews

- Thome, M. (2008) Multifunctional roles for MALT1 in T cell activation. Nature Rev. Immunol. (in press)
- Rebeaud, F., Hailfinger, S. and Thome, M. (2007) Dlgh1 and Carma1 MAGUK proteins contribute to signal specificity downstream of TCR activation. Trends Immunol. 28, 196-200.
- Thome, M. and Weil, R. (2007) Posttranslational modifications regulate distinct functions of Carma1 and Bcl10. Trends Immunol. 28, 281-288.
- Rueda, D. and Thome, M. (2005) Phosphorylation of Carma1: the link(er) to NF-kB activation. Immunity 23, 551-553
- Thome, M. (2004) Carma1, Bcl10 and Malt1 in lymphocyte development and activation. Nature Rev. Immunol. 4, 348-359.

Together, these results have led to the identification of two previously unsuspected, NF-kB independent functions of BCL-10 in the regulation of signaling pathways that are critical for changes in cell shape and adhesion (Fig. 1 and 3b). Further studies are aimed at the identification of the BCL-10 targets relevant for these pathways, and at the further elucidation of the physiological relevance of these observations.

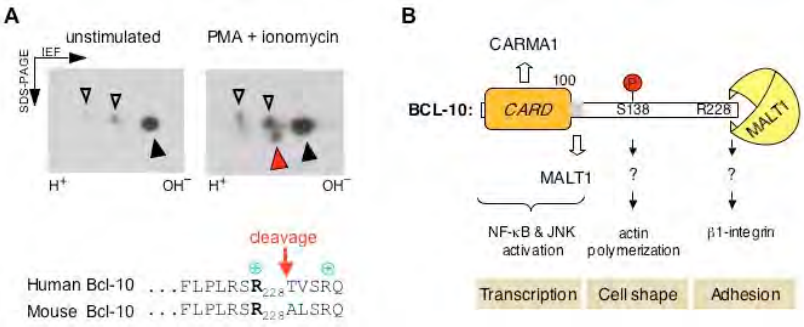


***Figure 3**: **T-cell activation induces phosphorylation and cleavage of BCL-10 to control various aspects of T-cell activation. (A)** Analysis of BCL-10 from unstimulated or activated T cells by 2-dimensional gel electrophoresis, which separates proteins according to size (SDS-PAGE) or isoelectric point (IEF). T-cell activation with PMA and ionomycin induces phosphorylation of BCL-10 on two sites (indicated by open arrowheads) and C-terminal cleavage of BCL-10 after Arginine 228 (red arrowhead and arrow), which leads to acidification of the protein because of removal of the positively charged Arginine residue 232 (lower panel). The position of unmodified BCL-10 is indicated by a black arrowhead. **(B)** BCL-10 has multiple functions in T cells: binding to CARMA1 (via the CARD motif) and to MALT1 is critical for NF-κB and JNK activation and the control of TCR-induced transcription (Gaide et al., 2002), Ser 138-dependent phosphorylation is required for TCR-induced actin polymerization and actin-dependent changes in cell shape (Rueda et al., 2006), while MALT1-dependent cleavage close to the C-terminus of BCL-10, after Arginine 228, is relevant for beta1-integrin-mediated adhesion of T-cells (Rebeaud, Hailfinger et al., 2008).*

Collaborations

Parts of this work were done in collaboration with the laboratories of Nicolas Fasel (Department of Biochemistry of the University of Lausanne), Nathalie Rufer (Multidisciplinary Oncology Center, University Hospital of Lausanne), Jürg Schwaller (formerly at the University Hospital of Geneva), Florence Niedergang (Institut Cochin, Paris) and Jérôme Delon (Institut Cochin, Paris).

Jürg Tschopp, Professor

Jürg Tschopp received his PhD in biophysics at the University of Basel in 1979. He then joined the group of Müller-Eberhard at the Scripps Clinic in La Jolla. In 1982, he was appointed assistant professor at the Department of Biochemistry of the University of Lausanne, where he was promoted to the rank of full professor in 1989. Since 2003 he is deputy-director of the Department of Biochemistry. His present research focuses on signaling pathways that control apoptosis and innate immunity.

Pathogen- and danger-sensing platforms that trigger apoptosis or innate immunity

Apoptosis is a naturally occurring process of cell death. All mammalian cells constitutively express the basic machinery that mediates apoptotic cell death, including a family of cysteine proteases, designated the caspases. Pro-apoptotic caspases are generally activated by death-receptors or damaged mitochondria and are inhibited by a number of cytoplasmic proteins including the caspase-8 homologue FLIP. Modulators of caspase activation are aberrantly expressed in pathological processes such as neurodegenerative diseases or cancer.
In contrast to caspase-8, the inflammatory caspase-1 is not involved in apoptosis but in the proteolytic activation of IL-1β, which triggers the rapidly acting innate immune system. Caspase-1 is activated by a complex, called inflammasome, which comprises NALP3, ASC and caspase-1. The importance of the NALP3 inflammasome in the process of inflammation is underscored by the observation that mutations of the NALP3 gene are associated with several autoinflammatory diseases. While the inflammasome is activated by bacteria and danger signals such as uric acid crystals, another cytoplasmic complex, called PIDDosome, detects DNA damage and orchestrates DNA repair. If unsuccessful, PIDD, the scaffolding protein of the PIDDosome, recruits caspase-2 and initiates cell death. Yet another cytoplasmic complex which is studied in our group is formed by RIG-I and Cardif. RIG-I-Cardif senses the presence of viral RNA and triggers an innate anti-viral response including the synthesis of type I interferon.
Our goal is to understand the signaling networks that control these inflammatory and apoptotic responses to pathogens and danger signals. We believe that this will provide important insights into the genesis of various human diseases.

The inflammasome: A platform sensing PAMPs and danger-associated molecules triggering innate immunity

The inflammasome is a multiprotein complex responsible for the activation of caspase-1 and -5, thereby leading to the activation of the pro-inflammatory cytokines IL-1β and IL-18. Our group identified two types of inflammasomes; the NALP1-inflammasome, which is composed of NALP1/ASC/Caspase-1/Caspase-5 and the NALP2/3-inflammasomes that contain, in addition to NALP2 or NALP3, CARDINAL/ASC/Caspase-1 (Fig. 1). An alternative inflammasome is formed by IPAF that directly recruits caspase-1 without the need of an adaptor protein.

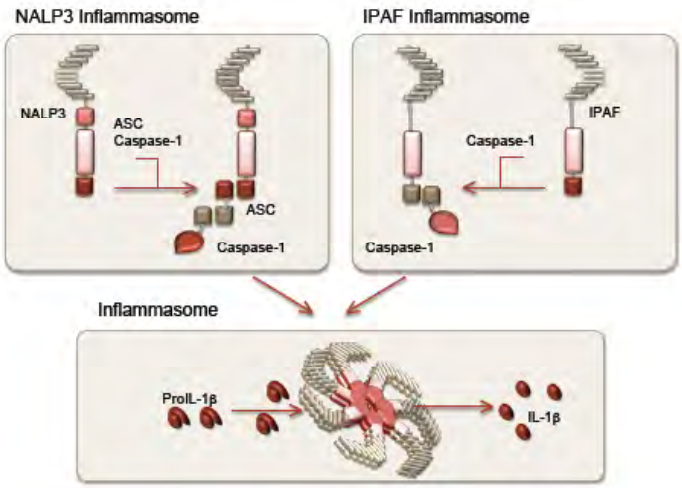


***Figure 1**: The inflammasomes*
Structural organization of the typical NALP3 and IPAF inflammasomes. The core structure of the NALP3 inflammasome is formed by NALP3, the adaptor ASC, and caspase-1 (left panel). IPAF recruits caspase-1 directly via CARD–CARD interactions (right panel). The LRR of NALP3 or IPAF sense the activating signals leading to the oligomerization of the NACHT region and initiating the formation of the donut-shaped inflammasome. Based on the structure of the apoptosome, the caspases and IL-1β-processing activity most likely face the inside of the donut (lower panel).

Selected publications

- Muruve, D.A., Petrilli, V., Zaiss, A.K., White, L.R., Clark, S.A, Ross' J., Parks, R.A. and Tschopp, J. (2008) The inflammasome recognizes cytosolic microbial and host DNA and triggers an innate immune response. Nature, 452, 103-107.
- Dostert, C., Petrilli, V., Van Bruggen, R., Steele, C., Mossman, B.T. and Tschopp, J. (2008) Innate immune activation through Nalp3 inflammasome sensing of asbestos and silica. Science 320, 674-677.
- Michallet, M.C., Meylan, E., Ermolaeva, M.A., Vazquez, J., Rebsamen, M., Curran, J., Poeck, H., Bscheider, M., Hartmann, G., Konig, M., et al. (2008). TRADD protein is an essential component of the RIG-like helicase antiviral pathway. Immunity 28, 651-661.
- Park, H.H., Logette, E., Raunser, S., Cuenin, S., Walz, T., Tschopp, J. and Wu, H. (2007) Death Domain Assembly Mechanism Revealed by Crystal Structure of the Oligomeric PIDDosome Core Complex. Cell 128, 533-546.
- Mayor, A., Martinon, F., De Smedt, T., Petrilli, V. and Tschopp, J. (2007) A crucial function of SGT1 and HSP90 in inflammasome activity links mammalian and plant innate immune responses. Nat. Immunol. 8, 497-503.
- *Meylan, E., Tschopp, J., and Karin, M. (2006). Intracellular pattern recognition receptors in the host response. Nature *442*, 39-44.
- Martinon, F., Petrilli, V., Mayor, A., Tardivel, A., and Tschopp, J. (2006). Gout-associated uric acid crystals activate the NALP3 inflammasome. Nature. *440*, 237-241.
- Gurcel, L., Abrami, L., Girardin, S., Tschopp, J., and van der Goot, F. G. (2006). Caspase-1 activation of lipid metabolic pathways in response to bacterial pore-forming toxins promotes cell survival. Cell *126*, 1135-1145.
- Meylan, E., Curran, J., Hofmann, K., Moradpour, D., Binder, M., Bartenschlager, R. and Tschopp, J. (2005) Cardif is an adaptor protein in the RIG-I antiviral pathway and is targeted by hepatitis C virus. Nature 437, 1167-1172.
- Janssens, S., Tinel, A., Lippens, S. and Tschopp, J. (2005) PIDD mediates NF-kappaB activation in response to DNA damage. Cell 123, 1079-1092.

The NALPs and IPAF are central proteins in the inflammasome complex. They belong to the NLR (NOD-like receptors) family of cytoplasmic proteins. Fourteen NALP proteins have been identified in humans. The role of most of these proteins remains to be determined. Little is also known about the natural stimuli that lead to the assembly and activation of the inflammasomes. Similar to Toll-like receptors, activation of the inflammasome is thought to occur through the recognition of pathogen-associated patterns (PAMPs) by the Leucine-Rich Repeats (LRR) present in the NALP proteins. For example muramyl dipeptide (MDP), a component of peptidoglycans, which is a cell wall component present in both Gram-positive and Gram-negative bacteria, activates the inflammasome.

While inflammasomes are emerging more and more as key players of the inflammatory and immune responses, a growing number of studies also reveals their function in the sensing of a controversial signal in immunology: danger. Indeed, activation of the NALP3 inflammasome is also induced by endogenous, microbe-independent stress signals. For instance, the exposure of macrophages to ATP, monosodiumurate (MSU) crystals or asbestos induces strong activation of caspase-1 in a NALP3 inflammasome-dependent manner.

Inflammasome and diseases

Mutations in the gene coding for NALP3 have been associated with several autoinflammatory disorders such as Muckle-Wells syndrome, familial cold urticaria and CINCA (Chronic Infantile Neurological Cutaneous and Articular autoinflammatory disease). These disorders are characterized by recurrent episodes of fever and serosal inflammation, due to increased production of IL-1β. Based on the discovery of the function of the inflammasome, patients are now successfully treated with the natural IL-1 inhibitor IL-1ra (Anakinra). Development of the acute and chronic inflammatory responses known as gout and pseudogout are associated with the deposition of monosodium urate (MSU) or calcium pyrophosphate dihydrate (CPPD) crystals, respectively, in joints and periarticular tissues (Fig. 2).

***Figure 2**: "The Gout" by James Gilray, 1799: gout depicted as an evil demon attacking a toe.*

Although MSU crystals were first identified as the etiologic agent of gout in the 18th century and more recently as a "danger signal" released from dying cells, little is known concerning the molecular mechanisms underlying MSU- or CPPD-induced inflammation. We found that MSU and CPPD engage the NALP3 inflammasome, resulting in the production of active IL-1β and IL-18. Macrophages from mice deficient in various components of the inflammasome such as caspase-1, ASC and NALP3 are defective in crystal-induced IL-1β activation. Moreover, an impaired neutrophil influx is found in an *in vivo* model of crystal-induced peritonitis in inflammasome-deficient mice or mice deficient in the IL-1β receptor (IL-1R). These findings further support a pivotal role of the inflammasome in several autoinflammatory diseases.

The RIG-I/CARDIF platform: Sensing viruses and activating type I interferons

Innate immunity against a pathogen is mounted upon recognition by the host of, for example, viral products. One of these viral 'signatures', double-stranded (ds) RNA, is a replication product of most viruses within infected cells and is sensed by Toll-like receptor 3 (TLR3) and the recently identified cytosolic RNA helicases RIG-I and MDA5. Both helicases detect dsRNA, and through their protein-interacting CARD domains, relay a poorly defined signal resulting in the activation of the transcription factors interferon regulatory factor 3 (IRF3) and NF-κB. We have recently identified Cardif (also known as IPS-1, MAVS or VISA), a

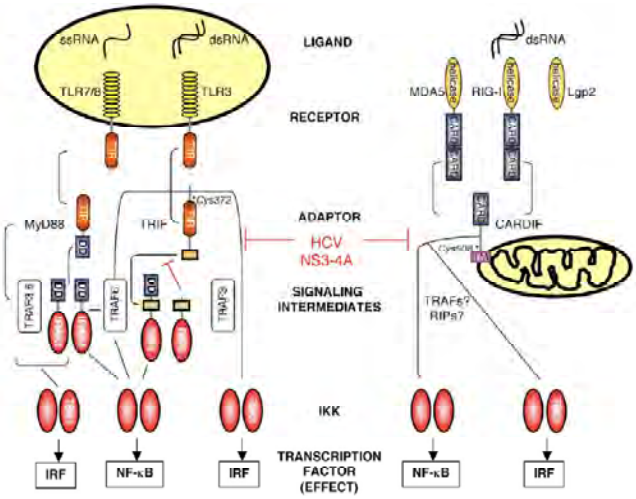


***Figure 3**: Overview of TLR- and RIG-I-dependent antiviral pathways (Left) Upon ssRNA or dsRNA binding within endosomes, TLR7/8 and TLR3, respectively, recruit the adaptors MyD88 or Trif through a TIR-TIR interaction. MyD88 recruits IRAKs through DD-DD interactions, and also TRAF3 and TRAF6, to activate IRF and NF-kB. Trif recruits RIP1 through a RHIM-RHIM interaction to activate NF-kB. To activate IRF, Trif recruits TBK1 and possibly TRAF3. (Right) Upon dsRNA binding, RIG-I (and also MDA5) recruits the mitochondrial-bound adaptor Cardif, which in turn recruits appropriate IKKs to activate NF-kB and IRF. During an infection with HCV, both Trif and Cardif are cleaved by the HCV NS3-4A protease. As a consequence, Trif cannot recruit TBK1, which prevents IRF activation and type-I interferon production.*

new CARD-containing adaptor protein that interacts with RIG-I and recruits IKKα, IKKβ and IKKε kinases by means of its C-terminal region, leading to the activation of NF-κB and IRF3. Overexpression of Cardif results in interferon-β and NF-κB promoter activation, and knockdown of Cardif inhibits RIG-I-dependent antiviral responses. Cardif is localized to mitochondria and is targeted and inactivated by NS3-4A, a serine protease from hepatitis C virus known to block interferon-β production. Cardif thus functions as an adaptor, linking the cytoplasmic dsRNA receptor RIG-I to the initiation of antiviral programmes.

TNF receptor and Cardif-induced death and survival signals are similar

Binding of TNF to the TNFR1 results in the generation of two opposite signals leading to NF-κB activation and apoptosis.

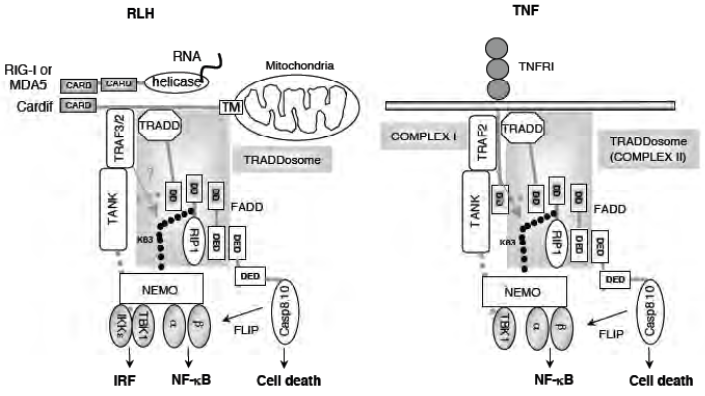


***Figure 4**: A schematic overview of the signaling pathways triggered by RLH-Cardif and TNFRI. Viral RNA binds to RIG-I or MDA5, which transmit the downstream signals via Cardif to activate IRF3 and NF-kB. A central signaling unit is the TRADDosome which consists of TRADD-RIP1 and FADD and which is also crucial for TNFR1-mediated proinflammatory responses.*

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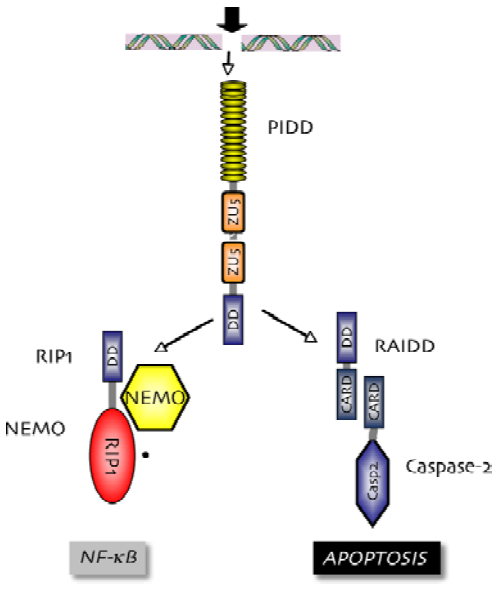

***Figure 5**: PIDD acts as a molecular switch, controlling the balance between life and death upon DNA damage.*

The initial plasma membrane-bound complex (complex I) consists of TNFR1, TRADD, RIP1 and TRAF2, and rapidly signals activation of the transcription factor NF-κB (Fig. 4). In this complex, TRADD and RIP1 undergo important posttranlational modifications and subsequently dissociate from the receptor. In a second step, TRADD and RIP1 associate with FADD and caspase-8, thereby forming the cytoplasmic complex II. In surviving cells where NF-κB is activated by complex I, complex II harbors the caspase-8 inhibitor FLIPL. In apoptosis-sensitive, NF-κB signal-defective cells, substantial amounts of caspase-10 are found in complex II while FLIPL levels are highly reduced. Thus, TNFR1-triggered signal transduction includes a check-point, resulting in cell death (via signal complex II) in instances where the initial signal (via complex I, NF-κB) fails to be activated.

Upon detection of viral RNA, the helicases RIG-I and/or MDA5 trigger, via their adaptor Cardif, the activation of the transcription factors NF-κB and IRF3, which collaborate to induce an antiviral type I interferon response (see above). Interestingly, FADD, TRADD and RIP1 are also implicated in the antiviral pathway triggered by Cardif and RIG-I (see above). TRADD is recruited to Cardif and orchestrates complex formation with the E3 ubiquitin ligase TRAF3 and TANK, and with FADD and RIP1, leading to the activation of IRF3 and NF-kB. Loss of TRADD prevents Cardif-dependent activation of interferon-β, reduces the production of interferon-β in response to RNA viruses, and enhances vesicular stomatitis virus replication. Thus, TRADD is not only an essential component of pro-inflammatory TNFRI signaling, but is also required for RLH-Cardif-dependent antiviral immune responses.

The PIDDosome: Detecting DNA damage and activating caspase-2

Activation of initiatior caspases is a key event in apoptosis execution. Prototypically, activation is triggered upon complex-mediated clustering of the inactive zymogen such as in the caspase-9-activating apoptosome complex. Likewise, caspase-2, which is involved in stress-induced apoptosis, is recruited into a large protein complex that contains the death-domain containing protein PIDD. Increased amounts of PIDD expression result in spontaneous activation of caspase-2 and sensitization to apoptosis by genotoxic stimuli. Because PIDD functions in p53-mediated apoptosis, the complex assembled by PIDD and caspase-2 is likely to have a crucial role in the regulation of apoptosis induced by genotoxins. We found that PIDD also plays a critical role in DNA damage-induced NF-κB activation. Upon genotoxic stress, a complex between PIDD, RIP1 and NEMO is formed. Cells stably expressing PIDD show enhanced genotoxic stress-induced NF-κB activation, through augmented sumoylation and ubiquitination of NEMO. Knock-down of PIDD and RIP1 expression abrogates DNA damage-induced NEMO-modification and hence NF-κB activation.

PIDD is constitutively processed giving rise to a 48-kDa N-terminal fragment and a 51-kDa C-terminal fragment (PIDD-C). The latter undergoes further cleavage resulting in a 37-kDa fragment (PIDD-CC). Processing occurs at S446 (generating PIDD-C) and S588 (generating PIDD-CC) by an auto-processing mechanism. Auto-cleavage of PIDD determines the outcome of the downstream signaling events. Whereas initially formed PIDD-C mediates the activation of NF-κB via the recruitment of RIP1 and NEMO, subsequent formation of PIDD-CC causes caspase-2 activation and thus cell death. In this way, auto-proteolysis of PIDD might participate in the orchestration of the DNA damage-induced life and death signaling pathways (Fig. 5).

ARTICLES

1. Acestor, N., Masina, S., Ives, A., Walker, J., Saravia, N.G., and Fasel, N. (2006). Resistance to oxidative stress is associated with metastasis in mucocutaneous leishmaniasis. *J Infect Dis* 194, 1160-1167.
2. Allenbach, C., Zufferey, C., Perez, C., Launois, P., Mueller, C., and Tacchini-Cottier, F. (2006). Macrophages induce neutrophil apoptosis through membrane TNF, a process amplified by Leishmania major. *J Immunol* 176, 6656-6664.
3. Arrigoni, G., Resjo, S., Levander, F., Nilsson, R., Degerman, E., Quadroni, M., Pinna, L.A., and James, P. (2006). Chemical derivatization of phosphoserine and phosphothreonine containing peptides to increase sensitivity for MALDI-based analysis and for selectivity of MS/MS analysis. *Proteomics* 6, 757-766.
4. Ayyanan, A., Civenni, G., Ciarloni, L., Morel, C., Mueller, N., Lefort, K., Mandinova, A., Raffoul, W., Fiche, M., Dotto, G. P., and Brisken, C. (2006). Increased Wnt signaling triggers oncogenic conversion of human breast epithelial cells by a Notch-dependent mechanism. *Proc Natl Acad Sci U S A* 103, 3799-3804.
5. Baars, T., Petri, S., Peters, C., and Mayer, A. (2007). Role of the V-ATPase in regulation of the vacuolar fission-fusion equilibrium. *Mol Biol Cell* 18, 3873-82.
6. Bongfen, S., Torgler, R., Romero, J., Renia, L., and Corradin, G. (2007). Plasmodium berghei-infected primary hepatocytes process and present the circumsporozoite protein to specific CD8+ T cells in vitro. *J Immunol* 178, 7054-6.
7. Bossen, C., Tardivel, A., Cachero, T.G., Scott, M.L., Tschopp, J., and Schneider, P. (2007). Baff multimerization is essential for signaling b cell survival through taci, but not baff-r. *Swiss Medical Weekly* 137, 17S-17S.
8. Bossen, C., Ingold, K., Tardivel, A., Bodmer, J.L., Gaide, O., Hertig, S., Ambrose, C., Tschopp, J., and Schneider, P. (2006). Interactions of tumor necrosis factor (TNF) and TNF receptor family members in the mouse and human. *J Biol Chem* 281, 13964-13971.
9. Bourreau, E., Ronet, C., Couppié, P., Sainte-Marie, D., Tacchini-Cottier, F., and Launois, P. (2007). IL-10 producing CD8+ T cells in human infection with Leishmania guyanensis. *Microbes Infect* 9, 1034-41.
10. Brissoni, B., Agostini, L., Kropf, M., Martinon, F., Swoboda, V., Lippens, S., Everett, H., Aebi, N., Janssens, S., Meylan, E., Felberbaum-Corti, M., Hirling, H., Gruenberg, J., Tschopp, J., and Burns, K. (2006). Intracellular trafficking of interleukin-1 receptor I requires Tollip. *Curr Biol* 16, 2265-2270.
11. Buchegger, F., Antonescu, C., Delaloye, A.B., Helg, C., Kovacsovics, T., Kosinski, M., Mach, J.P., and Ketterer, N. (2006). Long-term complete responses after 131I-tositumomab therapy for relapsed or refractory indolent non-Hodgkin's lymphoma. *Br J Cancer* 94, 1770-1776.
12. Cachero, T. G., Schwartz, I. M., Qian, F., Day, E. S., Bossen, C., Ingold, K., Tardivel, A., Krushinskie, D., Eldredge, J., Silvian, L., Lugovskoy, A., Farrington, G. K., Strauch, K., Schneider, P., and Whitty, A. (2006). Formation of virus-like clusters is an intrinsic property of the tumor necrosis factor family member BAFF (B cell activating factor). *Biochemistry (Mosc)* 45, 2006-2013.

13. Casal, M.L., Lewis, J.R., Mauldin, E.A., Tardivel, A., Ingold, K., Favre, M., Paradies, F., Demotz, S., Gaide, O., and Schneider, P. (2007). Significant correction of disease after postnatal administration of recombinant ectodysplasin a in canine x-linked ectodermal dysplasia. *American Journal of Human Genetics* 81, 1050-1056.
14. Cesson, V., Stirnemann, K., Robert, B., Luescher, I., Filleron, T., Corradin, G., Mach, J.P., and Donda, A. (2006). Active antiviral T-lymphocyte response can be redirected against tumor cells by antitumor antibody x MHC/viral peptide conjugates. *Clin Cancer Res* 12, 7422-7430.
15. Chapatte, L., Ayyoub, M., Morel, S., Peitrequin, A.L., Levy, N., Servis, C., Van den Eynde, B.J., Valmori, D., and Levy, F. (2006). Processing of tumor-associated antigen by the proteasomes of dendritic cells controls in vivo T-cell responses. *Cancer Res* 66, 5461-5468.
16. Charmoy, M., Megnekou, R., Allenbach, C., Zweifel, C., Perez, C., Monnat, K., Breton, M., Ronet, C., Launois, P., and Tacchini-Cottier, F. (2007). Leishmania major induces distinct neutrophil phenotypes in mice that are resistant or susceptible to infection. *J Leukoc Biol* 82, 288-99.
17. Corazza, N., Jakob, S., Schaer, C., Frese, S., Keogh, A., Stroka, D., Kassahn, D., Torgler, R., Mueller, C., Schneider, P., and Brunner, T. (2006). TRAIL receptor-mediated JNK activation and Bim phosphorylation critically regulate Fas-mediated liver damage and lethality. *J Clin Invest* 116, 2493-2499.
18. Corradin, G. (2007) Peptide based malaria vaccine development: personal considerations. *Microbes Infect* 9, 767-71.
19. Corradin, G., Villard, V., and Kajava, A. (2007). Protein structure based strategies for antigen discovery and vaccine development against malaria and other pathogens. *Endocr Metab Immune Disord Drug Targets* 7, 259-65.
20. Devgan, V., Nguyen, B.C., Oh, H., and Dotto, G.P. (2006). p21WAF1/Cip1 suppresses keratinocyte differentiation independently of the cell cycle through transcriptional up-regulation of the IGF-I gene. *J Biol Chem* 281, 30463-30470.
21. Didierlaurent, A., Brissoni, B., Velin, D., Aebi, N., Tardivel, A., Kaslin, E., Sirard, J.C., Angelov, G., Tschopp, J., and Burns, K. (2006). Tollip regulates proinflammatory responses to interleukin-1 and lipopolysaccharide. *Mol Cell Biol* 26, 735-742
22. Dostert, C., Meylan, E., and Tschopp, J. (2007). Intracellular pattern-recognition receptors. *Adv Drug Deliv Rev*
23. Farley, S.M., Dotson, A.D., Purdy, D.E., Sundholm, A.J., Schneider, P., Magun, B.E., and Iordanov, M.S. (2006). Fas Ligand Elicits a Caspase-Independent Proinflammatory Response in Human Keratinocytes: Implications for Dermatitis. *J Invest Dermatol* 126, 2438-2451.
24. Fattah, O.M., Cloutier, S.M., Kundig, C., Felber, L.M., Gygi, C.M., Jichlinski, P., Leisinger, H.J., Gauthier, E.R., Mach, J.P., and Deperthes, D. (2006). Peptabody-EGF: a novel apoptosis inducer targeting ErbB1 receptor overexpressing cancer cells. *Int J Cancer* 119, 2455-2463.

25. Genton, C., Wang, Y., Izui, S., Malissen, B., Delsol, G., Fournie, G. J., Malissen, M., and Acha-Orbea, H. (2006). The Th2 lymphoproliferation developing in LatY136F mutant mice triggers polyclonal B cell activation and systemic autoimmunity. *J Immunol* 177, 2285-2293.
26. Girgenrath, M., Weng, S., Kostek, C.A., Browning, B., Wang, M., Brown, S.A., Winkles, J.A., Michaelson, J.S., Allaire, N., Schneider, P., Scott, M.L., Hsu, Y.M., Yagita, H., Flavell, R.A., Miller, J.B., Burkly, L.C., and Zheng, T.S. (2006). TWEAK, via its receptor Fn14, is a novel regulator of mesenchymal progenitor cells and skeletal muscle regeneration. *EMBO J* 25, 5826-5839.
27. González, I., Desponds, C., Schaff, C., Mottram, J., and Fasel, N. (2007). Leishmania major metacaspase can replace yeast metacaspase in programmed cell death and has arginine-specific cysteine peptidase activity. *Int J Parasitol* 37, 161-72.
28. Gorczynski, R.M., Alexander, C., Bessler, W., Brandenburg, K., Fournier, K., Mach, J.P., Mueller, S., Rietschel, E.T., Ulmer, A.J., Waelli, T., Zahringer, U., and Khatri, I. (2007). An alteration in the levels of populations of CD4(+) Treg is in part responsible for altered cytokine production by cells of aged mice which follows injection with a fetal liver extract. *Immunology Letters* 109, 101-112.
29. Grutter, C., Briand, C., Capitani, G., Mittl, P.R., Papin, S., Tschopp, J., and Grutter, M.G. (2006). Structure of the PRYSPRY-domain: implications for autoinflammatory diseases. *FEBS Lett* 580, 99-106.
30. Guichard, G., Trouche, N., Wieckowski, S., Sun, W., Chaloin, O., Bianco, A., Hoebeke, J., Schneider, P., and Fournel, S. (2007). Rationally-designed multivalent architectures for mimicking homotrimers of CD40L, a member of the TNF superfamily. *Biopolymers* 88, 522-522.
31. Gumy, A., Aseffa, A., Rachinel, N., Breton, M., Otten, L., Tacchini-Cottier, F., Rocken, M., Doyen, N., Acha-Orbea, H., Locksley, R.M., MacDonald, H.R., Launois, P., and Louis, J. (2006). LACK-reactive CD4+ T cells require autocrine IL-2 to mediate susceptibility to Leishmania major. *Eur J Immunol* 36, 1465-1473.
32. Gurcel, L., Abrami, L., Girardin, S., Tschopp, J., and van der Goot, F. G. (2006). Caspase-1 activation of lipid metabolic pathways in response to bacterial pore-forming toxins promotes cell survival. *Cell* 126, 1135-1145.
33. Hermsen, C.C., Verhage, D.F., Telgt, D. S., Teelen, K., Bousema, J.T., Roestenberg, M., Bolad, A., Berzins, K., Corradin, G., Leroy, O., Theisen, M., and Sauerwein, R.W. (2006). Glutamate-rich protein (GLURP) induces antibodies that inhibit in vitro growth of Plasmodium falciparum in a phase 1 malaria vaccine trial. *Vaccine* 25, 2930-40
34. Kariminia, A., Bourreau, E., Ronet, C., Couppie, P., Sainte-Marie, D., Tacchini-Cottier, F., and Launois, P. (2007). Selective expression of the V beta 14 T cell receptor on Leishmania guyanensis--specific CD8+ T cells during human infection. *J Infect Dis* 195, 739-47.
35. Kothlow, S., I. Morgenroth, Y. Graef, K. Schneider, I. Riehl, P. Staeheli, P. Schneider and B. Kaspers (2007) Unique and conserved functions of B cell-activating factor of the TNF family (BAFF) in the chicken. *International Immunology*. 19, 203-215.

36. Kummer, J.A., Micheau, O., Schneider, P., Bovenschen, N., Broekhuizen, R., Quadir, R., Strik, M.C.M., Hack, C.E., and Tschopp, J. (2007). Ectopic expression of the serine protease inhibitor PI9 modulates death receptor-mediated apoptosis. *Cell Death and Differentiation* 14, 1486-1496.
37. Launois, P., Pingel, S., Himmelrich, H., Locksley, R., and Louis, J. (2007). Different epitopes of the LACK protein are recognized by V beta 4 V alpha 8 CD4+ T cells in H-2b and H-2d mice susceptible to Leishmania major. *Microbes Infect* 9, 1260-6.
38. Lefort, K., Mandinova, A., Ostano, P., Kolev, V., Calpini, V., Kolfschoten, I., Devgan, V., Lieb, J., Raffoul, W., Hohl, D., Neel, V., Garlick, J., Chiorino, G., and Dotto, G. (2007). Notch1 is a p53 target gene involved in human keratinocyte tumor suppression through negative regulation of ROCK1/2 and MRCKalpha kinases. *Genes Dev* 21, 562-77.
39. Link, A., Vogt, G., Favre, S., Britschgi, M., Acha-Orbea, H., Hinz, B., Cyster, J., and Luther, S. (2007). Fibroblastic reticular cells in lymph nodes regulate the homeostasis of naive T cells. *Nat Immunol* 8, 1255-65.
40. Loeuillet, C., Martinon, F., Perez, C., Munoz, M., Thome, M., and Meylan, P.R. (2006). Mycobacterium tuberculosis subverts innate immunity to evade specific effectors. *J Immunol* 177, 6245-6255.
41. Luther, S., Serre, K., Cunningham, A., Khan, M., Acha-Orbea, H., MacLennan, I., and Toellner, K. (2007). Recirculating CD4 memory T cells mount rapid secondary responses without major contributions from follicular CD4 effectors and B cells. *Eur J Immunol* 37, 1476-84.
42. Martinon, F., Petrilli, V., Mayor, A., Tardivel, A., and Tschopp, J. (2006). Gout-associated uric acid crystals activate the NALP3 inflammasome. *Nature* 440, 237-241.
43. Masina, S., Zangger, H., Rivier, D., and Fasel, N. (2007). Histone H1 regulates chromatin condensation in Leishmania parasites. *Exp Parasitol* 116, 83-7.
44. Mayor, A., Martinon, F., De Smedt, T., Pétrilli, V., and Tschopp, J. (2007). A crucial function of SGT1 and HSP90 in inflammasome activity links mammalian and plant innate immune responses. *Nat Immunol* 8, 497-503.
45. Meier, D., Bornmann, C., Chappaz, S., Schmutz, S., Otten, L., Ceredig, R., Acha-Orbea, H., and Finke, D. (2007). Ectopic lymphoid-organ development occurs through interleukin 7-mediated enhanced survival of lymphoid-tissue-inducer cells. *Immunity* 26, 643-54.
46. Merino, D., Lalaoui, N., Morizot, A., Schneider, P., Solary, E., and Micheau, O. (2006). Differential Inhibition of TRAIL-Mediated DR5-DISC Formation by Decoy Receptors 1 and 2. *Mol Cell Biol* 26, 7046-7055.
47. Mou, C., Jackson, B., Schneider, P., Overbeek, P.A., and Headon, D. J. (2006). Generation of the primary hair follicle pattern. *Proc Natl Acad Sci U S A* 103, 9075-9080.
48. Munding, C., Keller, M., Niklaus, G., Papin, S., Tschopp, J., Werner, S., and Beer, H.D. (2006). The estrogen-responsive B box protein: a novel enhancer of interleukin-1beta secretion. *Cell Death Differ* 13, 1938-1949.

49. Nguyen, B.C., Lefort, K., Mandinova, A., Antonini, D., Devgan, V., Della Gatta, G., Koster, M.I., Zhang, Z., Wang, J., di Vignano, A.T., Kitajewski, J., Chiorino, G., Roop, D.R., Missero, C., and Dotto, G.P. (2006). Cross-regulation between Notch and p63 in keratinocyte commitment to differentiation. *Genes Dev* 20, 1028-1042.
50. Ongusaha, P.P., Kim, H.G., Boswell, S.A., Ridley, A.J., Der, C.J., Dotto, G.P., Kim, Y.B., Aaronson, S.A., and Lee, S.W. (2006). RhoE is a pro-survival p53 target gene that inhibits ROCK I-mediated apoptosis in response to genotoxic stress. *Curr Biol* 16, 2466-2472.
51. Otten, L.A., Leibundgut-Landmann, S., Huarte, J., Kos-Braun, I.C., Lavanchy, C., Barras, E., Borisch, B., Steimle, V., Acha-Orbea, H., and Reith, W. (2006). Revisiting the specificity of the MHC class II transactivator CIITA in vivo. *Eur J Immunol* 36, 1548-1558.
52. Papin, S., Cuenin, S., Agostini, L., Martinon, F., Werner, S., Beer, H., Grütter, C., Grütter, M., and Tschopp, J. (2007). The SPRY domain of Pyrin, mutated in familial Mediterranean fever patients, interacts with inflammasome components and inhibits proIL-1beta processing. *Cell Death Differ* 14, 1457-66.
53. Pétrilli, V., Dostert, C., Muruve, D., and Tschopp, J. (2007). The inflammasome: a danger sensing complex triggering innate immunity. *Curr Opin Immunol* 19, 615-22.
54. Pétrilli, V., Papin, S., Dostert, C., Mayor, A., Martinon, F., and Tschopp, J. (2007). Activation of the NALP3 inflammasome is triggered by low intracellular potassium concentration. *Cell Death Differ* 14, 1583-9.
55. Prato, S., Fleming, J., Schmidt, C.W., Corradin, G., and Lopez, J.A. (2006). Cross-presentation of a human malaria CTL epitope is conformation dependent. *Mol Immunol* 43, 2031-2036.
56. Pummila, M., Fliniaux, I., Jaatinen, R., James, M.J., Laurikkala, J., Schneider, P., Thesleff, I., and Mikkola, M.L. (2007). Ectodysplasin has a dual role in ectodermal organogenesis: inhibition of Bmp activity and induction of Shh expression. *Development* 134, 117-125.
57. Rebeaud, F., Hailfinger, S., and Thome, M. (2007). Dlgh1 and Carma1 MAGUK proteins contribute to signal specificity downstream of TCR activation. *Trends Immunol* 28, 196-200.
58. Romero, J., Ibrahim, G., Renggli, J., Himmelrich, H., Graber, P., and Corradin, G. (2007). IL-12p40-independent induction of protective immunity upon multiple Plasmodium berghei irradiated sporozoite immunizations. *Parasite Immunol* 29, 541-8.
59. Rossi, D.C., Ballenegger, S., Ingold, K., Schneider, P., and Tschopp, J. (2007). Kino me siRNA screen of the human Fas pathway. *Swiss Medical Weekly* 137, 29S-29S.
60. Rueda, D., Gaide, O., Ho, L., Lewkowicz, E., Niedergang, F., Hailfinger, S., Rebeaud, F., Guzzardi, M., Conne, B., Thelen, M., Delon, J., Ferch, U., Mak, T., Ruland, J., Schwaller, J., and Thome, M. (2007). Bcl10 controls TCR- and FcgammaR-induced actin polymerization. *J Immunol* 178, 4373-84.
61. Schmidt-Ullrich, R., Tobin, D. J., Lenhard, D., Schneider, P., Paus, R., and Scheidereit, C. (2006). NF-kappaB transmits Eda A1/EdaR signalling to activate Shh and cyclin D1 expression, and controls post-initiation hair placode down growth. *Development* 133, 1045-1057.

62. Schwaller, J., Schneider, P., Mhawech-Fauceglia, P., McKee, T., Myit, S., Matthes, T., Tschopp, J., Donze, O., Le Gal, F.A., and Huard, B. (2007). Neutrophil-derived APRIL concentrated in tumor lesions by proteoglycans correlates with human B-cell lymphoma aggressiveness. *Blood* 109, 331-338.
63. Sharov, A.A., Sharova, T.Y., Mardaryev, A.N., di Vignano, A.T., Atoyan, R., Weiner, L., Yang, S., Brissette, J.L., Dotto, G.P., and Botchkarev, V.A. (2006). Bone morphogenetic protein signaling regulates the size of hair follicles and modulates the expression of cell cycle-associated genes. *Proc Natl Acad Sci U S A* 103, 18166-18171.
64. Thurau, M., Everett, H., Tapernoux, M., Tschopp, J., and Thome, M. (2006). The TRAF3-binding site of human molluscipox virus FLIP molecule MC159 is critical for its capacity to inhibit Fas-induced apoptosis. *Cell Death Differ* 13, 1577-1585.
65. Tinel, A., Janssens, S., Lippens, S., Cuenin, S., Logette, E., Jaccard, B., Quadroni, M., and Tschopp, J. (2007). Autoproteolysis of PIDD marks the bifurcation between pro-death caspase-2 and pro-survival NF-kappaB pathway. *EMBO J* 26, 197-208.
66. Uttenweiler, A., Schwarz, H., Neumann, H., and Mayer, A. (2007). The vacuolar transporter chaperone (VTC) complex is required for microautophagy. *Molecular Biology of the Cell* 18, 166-175.
67. Villard, V., Agak, G., Frank, G., Jafarshad, A., Servis, C., Nébié, I., Sirima, S., Felger, I., Arevalo-Herrera, M., Herrera, S., Heitz, F., Bäcker, V., Druilhe, P., Kajava, A., and Corradin, G. (2007). Rapid identification of malaria vaccine candidates based on alpha-helical coiled coil protein motif. *PLoS ONE* 2, e645.
68. Villard, V., Kalyuzhniy, O., Riccio, O., Potekhin, S., Melnik, T.N., Kajava, A.V., Ruegg, C., and Corradin, G. (2006). Synthetic RGD-containing alpha-helical coiled coil peptides promote integrin-dependent cell adhesion. *J Pept Sci* 12, 206-212.
69. Vince, J.E., Wong, W.W.L., Khan, N., Feltham, R., Chau, D., Ahmed, A.U., Benetatos, C.A., Chunduru, S.K., Condon, S.M., McKinlay, M., Brink, R., Leverkus, M., Tergaonkar, V., Schneider, P., Callus, B.A., Koentgen, F., Vaux, D.L., and Silke, J. (2007). IAP antagonists target cIAP1 to induce TNF alpha- dependent apoptosis. *Cell* 131, 682-693.
70. Walker, J., Acestor, N., Gongora, R., Quadroni, M., Segura, I., Fasel, N., and Saravia, N.G. (2006). Comparative protein profiling identifies elongation factor-1beta and tryparedoxin peroxidase as factors associated with metastasis in Leishmania guyanensis. *Mol Biochem Parasitol* 145, 254-264.
71. Willett, B.J., McMonagle, E.L., Logan, N., Spiller, O.B., Schneider, P., and Hosie, M.J. (2007). Probing the interaction between feline immunodeficiency virus and CD134 by using the novel monoclonal antibody 7D6 and the CD134 (OX40) ligand. *Journal of Virology* 81, 9665-9679.

REVIEWS

1. Acha-Orbea, H., Shakhov, A., and Finke, D. (2007). Immune response to MMTV infection. *Front Biosci* 12, 1594-609.
2. Bossen, C., and Schneider, P. (2006). BAFF, APRIL and their receptors: Structure, function and signaling. *Semin Immunol* 18, 263-275.
3. Budd, R.C., Yeh, W.C., and Tschopp, J. (2006). cFLIP regulation of lymphocyte activation and development. *Nat Rev Immunol* 6, 196-204.
4. Janssens, S., and Tschopp, J. (2006). Signals from within: the DNA-damage-induced NF-kappaB response. *Cell Death Differ* 13, 773-784.
5. Martinon, F. and J. Tschopp (2007). Inflammatory caspases and inflammasomes: master switches of inflammation. *Cell Death Differ* 14, 10-22.
6. Meylan, E., and Tschopp, J. (2006). Toll-like receptors and RNA helicases: two parallel ways to trigger antiviral responses. *Mol Cell* 22, 561-569.
7. Meylan, E., Tschopp, J., and Karin, M. (2006). Intracellular pattern recognition receptors in the host response. *Nature* 442, 39-44.
8. Pétrilli, V., and Martinon, F. (2007). The inflammasome, autoinflammatory diseases, and gout. *Joint Bone Spine* 74, 571-6.
9. Thome, M., and Weil, R. (2007). Post-translational modifications regulate distinct functions of CARMA1 and BCL10. *Trends Immunol* 28, 281-8.

BOOK SECTIONS

1. Acha-Orbea, H. (2007). Rolf Zinkernagel, Professor für experimentelle Immunologie und Nobelpreisträger für Medizin "Ohne den Willen sich zu informieren, verbauen wir unsere Zukunft". *Swiss Guest Book.* Pfändler, B. (ed.), pp 118-121, Orell Füssli Verlag Zürich
2. Thome, M. (2006). Regulation of Fas signaling by FLIP proteins. In *Fas signaling*, H. Wajant, ed. (New York, Landes Bioscience, Springer Science + Business Media), pp. 38-50.