UNIL | Université de Lausanne
Faculté de biologie et de médecine

DEPARTEMENT DE BIOCHIMIE
RAPPORT 2004-2005

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Couverture: Organization of lymphocytes and stromal cells in secondary and tertiary lymphoid tissues of mice as shown by immunofluorescence staining of frozen tissue sections. The large panel shows B220+ B cell zone (blue) and gp38+ stromal cells (red) restricted to the T zone. In contrast, desmin+ stromal cells (green) are localized in T and B zones as well as the red pulp. The small panels show from top to bottom: 1) Insulin producing islet (red) disrupted by infiltrating B220+ B cells (green) and T cells (not shown) in a transgenic mouse model (RIP-BLC). The lymphoid compartments formed in these so called tertiary lymphoid tissues are very similar to the ones of secondary lymphoid tissues. 2) Splenic white pulp with CD3+ T cells (red) and CD8+ cytotoxic T cells (green). CD4+3+ T cells appear in red and CD8+3+ T cells in yellow with both subsets occupying the T zone. 3) Staining of the splenic white pulp for CD3+ T cells (red) and B220+ B cells (green) showing the distinct compartmentalization into T and B zones that is achieved by stromal cell-derived chemokines. Courtesy of Stéphanie Zutter and Sanjiv Luther.

Le dernier rapport d'activité biennal reflétait les profonds changements intervenus au sein du corps professoral du Département de Biochimie. Cinq professeurs se sont en effet retirés presque simultanément entre 2000 et 2003. Les années 2004-2005 correspondent à une période de consolidation en interne des activités du Département.

Les groupes des Professeurs Gian-Paolo Dotto et Andreas Mayer se sont bien intégrés. Il en est de même pour les groupes des trois Professeurs boursiers FNRS, Angelos Constantinou, Sanjiv Luther et Margot Thome. Les interactions avec le Centre OMS de recherche et de formation en immunologie dirigé par le Professeur Pascal Launois sont toujours aussi bonnes, et nombreux sont les membres du Département qui participent aux cours organisés par ce Centre. La direction se réjouit d'une telle collaboration et espère lui donner une plus grande visibilité au sein de la Faculté de Biologie et de Médecine.

Au chapitre des départs, nous nous devons de rappeler le départ à la retraite du Dr Denis Rivier qui a travaillé comme Maître d'enseignement et de recherche au sein de notre Département pendant plus de 30 ans. Il a soulagé nombre d'enseignants des tâches d'organisation des travaux pratiques et des examens. Au nom de tous les collaborateurs du Département, nous tenons à exprimer notre profonde gratitude à Denis Rivier pour le travail accompli pendant toutes ces années.

Les thématiques de recherche se sont focalisées dans le domaine du cancer et de l'immunologie. Des progrès importants ont été accomplis dans la mise en place d'un modèle de souris pour étudier l'histiocytose (Hans Acha-Orbea), la compréhension des voies de signalisation essentielles pour la croissance et la différenciation des kératinocytes (Gian-Paolo Dotto), les mécanismes de fusion des membranes cellulaires (Andreas Mayer), l'identification des ligands et des récepteurs pour le TNF (Pascal Schneider), la compréhension des signaux importants pour l'immunité innée et la mort cellulaire (Jürg Tschopp), les mécanismes de réparation et d'instabilité du génome (Angelos Constantinou), l'identification et le développement de vaccins (Giampietro Corradin), l'immunothérapie des tumeurs (Jean-Pierre Mach), la définition de facteurs de virulence (Nicolas Fasel) et la réponse immunitaire contre le parasite Leishmania (Pascal Launois et Fabienne Tacchini-Cottier), la fonction des cellules stromales dans les tissus lymphoïdes secondaires (Sanjiv Luther) et les mécanismes d'activation et de survie des lymphocytes T (Margot Thome Miazza). Certains de ces travaux ont été récompensés, notamment ceux du groupe de Hans Acha Orbea par le Histiocytosis Research Trust (HRT), UK, alors que Pascal Schneider a reçu le Prix Serono pour Jeune Chercheur 2005.

Les nombreuses publications de qualité démontrent qu'une fois encore le Département a maintenu son niveau d'excellence. C'est certainement dû à la volonté, exprimée par tous les chefs de groupe, de travailler ensemble pour maintenir ce niveau de qualité, d'accepter de partager des ressources restreintes et de profiter de l'environnement scientifique du site et du Département.

Le financement du Département est assuré à peu près à part égale entre l'Université et des fonds extérieurs. Cet élément montre aussi la détermination des membres du Département à être compétitifs au niveau international.

Les 7 thèses de doctorat soutenues durant ces deux dernières années, les 114 publications parues souvent dans des journaux à fort impact, l'importance des apports financiers extrabudgétaires pour conduire les programmes de recherche et finalement, le rôle déterminant joué par le Département dans la Faculté de Biologie et de Médecine sont autant de signes de sa vitalité. Certes, ceci résulte des compétences réunies de tous les collaborateurs, mais surtout du privilège de pouvoir conduire les

missions de recherche au sein d'un consortium d'instituts avec des intérêts scientifiques convergents. La réorganisation du centre de recherche d'Epalinges après le départ de l'Institut Suisse de Recherche Expérimentale sur le Cancer placera le Département de Biochimie dans une situation incertaine et peu propice au maintien de sa productivité et à son développement. Le Département tient à ce qu'une masse critique scientifique optimale soit assurée. Au moment de la rédaction de ce rapport, la Direction du Département, ainsi que les différents chefs de groupe, sont soucieux de leur avenir: aucun partenaire localisé sur le site d'Epalinges n'est identifié pour l'heure, les ressources publiques n'augmentent pas, l'espace attribué à la Biochimie limite son développement et l'arrivée de nouveaux groupes, les services communs aux différents Instituts du site pourraient être démantelés. Sans céder à la panique ni être polémiques à outrance, nous insistons pour qu'une solution soit trouvée rapidement. Des réponses à notre interpellation ne peuvent être que bénéfiques, non seulement pour notre Département, mais aussi pour l'ensemble de la recherche et de la formation dans les biosciences de l'arc lémanique.

N. Fasel et J. Tschopp

Personnel rétribué par l'UNIL: 54.85 EPT (équivalent plein temps)
* Personnel rétribué par des fonds privés: 35.35 EPT

1. DEPARTEMENT DE BIOCHIMIE (décembre 2005)

• 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
J.P. Kraehenbuhl	Professeur honoraire
S. Luther *	Professeur assistant (boursier FNS)
J.P. Mach	Professeur honoraire
J. Mauël	Professeur honoraire
A. Mayer	Professeur
D. Muruve *	Hôte sabbatique
P. Schneider	Professeur assistant
M. Thome Miazza *	Professeur assistant (boursier FNS)
J. Tschopp	Professeur

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S. Lippens	Maître-assistant suppléant
S. Masina	Maître-assistant suppléant
L. Otten	Maître-assistant suppléant
C. Peters	Maître-assistant

• Assistant(s)

Postdoctorant(e)s

M. Bianchi *	A. Donda *	H. Everett *
L. Franzini Brunner	S. Grossi *	I. Kolfschoten
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J. Romero *	D. Rueda-Fernandez	Q.G. Steiner *
A. Tinel	V. Villard	

Doctorant(e)s

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L. Michaillat	R. Moser	H. Neumann
F. Rebeaud *	E. Silayuv	K. Stirnemann *
V. Swoboda	M. Tapernoux	R. Torgler
A. Uttenweiler	T. Vogt *	J. Wagner

• Personnel technique

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C. Desponds *	K. Fournier *	G. Frank *
M. Guzzardi *	S. Hertig	K. Ingold *
C. Lavanchy *	A. Luther *	C. Mattmann
F. Prevel *	M. Reinhardt	L. Rodrigues *
M. Rousseaux	E. Säuberli *	S. Streuli *
A. Tardivel *	J. Vazquez *	S. Zutter *

Aide laborantine

L. Morgado *

Apprenti(e)s laborantin(e)s

A. Da Costa O. Froelicher C. Pavlin

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Atelier

D. Roy

Bibliothèque

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Laverie

L. Skupienski

Magasin central et Travaux pratiques

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P. Margot
U. Margot (50%)

Secrétariat

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F. Flejszman (75%)
M. Jayet Herzstein

2. CENTRE OMS DE RECHERCHE ET DE FORMATION EN IMMUNOLOGIE

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F. Tacchini-Cottier *	Privat-docent et MER
C. Ronet	Postdoctorante
C. Allenbach *	Doctorante
M. Breton	Doctorante
Y. Hauyon La Torre *	Laborantine
F. Flejszman *	Secrétaire

3. PLATEFORMES

- **Protein Analysis Facility**

M. Quadroni *	Maître d'enseignement et de recherche
W. Bienvenut *	Collaborateur scientifique
Thoppae Gnanasekaran	Bioinformaticien
B. Jaccard	Doctorante
J. Barblan *	Laborantin
A. Potts *	Laborantine

- **Protein and Peptide Chemistry Facility**

C. Servis*	Cheffe de projet
Nicole Lévy *	Laborantine
Florela Penea	Laborantine

4. DEPARTS

- **Corps enseignant**

D. Rivier	retraite (11.2005)	K. Burns Di Marco	(08.2005)

- **Assistants**

Postdoctorant(e)s

E. Arnaud	(11.2005)	E. Gärdby	(03.2004)
S. Janssens	(09.2005)	C. Mammucari	(02.2005)
F. Martinon	(12.2005)	S. Rybtsov	(02.2005)
M. Thurau	(08.2004)		

Thèses de doctorat

N. Acestor	(08.2005)	T. Andresen	(12.2004)
A. Bah	(07.2004)	A. Hiou	(09.2005)
H. Jaccard	(10.2005)	S. Monnerat	(04.2004)
C. Reese	(09.2005)	A. Tinel	(12.2005)

- **Personnel technique**

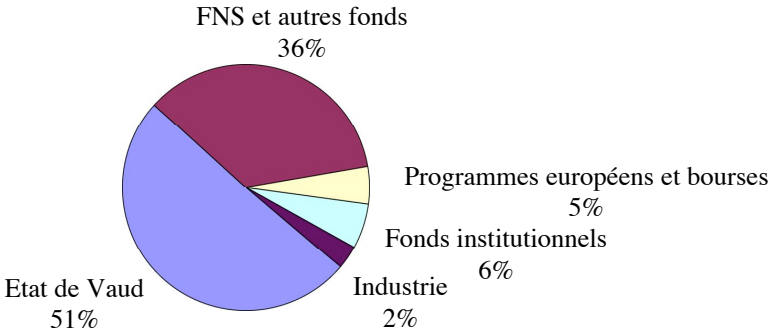
Laborantin(e)s

N. Aebi	(novembre 2005)	L. Bagnoud	(avril 2004)
A. Ransijn	(mars 2005)		

Apprenti laborantin

O. Gaudard (août 2004)

5. RESSOURCES 2005



Etat de Vaud/UNIL: CHF 5'739'859
Ressources externes: CHF 5'606'340

ENSEIGNEMENTS ET THESES DE DOCTORAT

ENSEIGNEMENTS

	Nombre d'heures/an				Etudiants	Nombre étudiants
	C	S	APP	TP		
1ère année						
Cellule	40	2	-	-	médecine	339
					biologie	128
2ème année						
Cellule	30	-	8	96	médecine	176
Sang,	21	-	22	-	médecine	176
immunité	34	6	8	-	médecine	176
Métabolisme	42	-	-	-	biologie	57
Métabolisme						
3ème année						
Bachelor	54	-	-	40	biologie	39
4ème année						
Master	86	8	-	116	biologie	9
	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 avancé. Epalinges, 13.05 - 25.06.2004
  - Surveillance des maladies transmissibles. Epalinges 28.06 - 09.07.2004
- Immunology, Vaccinology and Biotechnology applied to infectious diseases. 18 participants.
  - Advanced course. Epalinges, 08.09 - 24.10.2004
  - Refresher course. Cuba, 08.12 - 17.12.2004
  - Refresher course. Niamey, Niger, 28.02 - 16.03.2005
  - Advanced course. Epalinges, 07.09 - 22.10.2005
  - Surveillance of communicable diseases. Epalinges 24.10 - 04.11.2005

THESES DE DOCTORAT

Séverine Monnerat: Genomic organisation, gene expression and antisense transcription in a chromosomal region of *Leishmania major*.

Nathalie Acestor: The use of proteomics to identify markers associated with metastasis in *Leishmania Viannia* species: the role of tryparedoxin peroxidase.

Brian Brissoni: Characterization of Tollip in the IL-1R/TLR signaling pathways.

Heinz Neumann: Structure and function of the Vtc complex. (Université de Tübingen)

Christoph Reese: Lipid dynamics in intracellular membrane fusion. (Université de Tübingen)

Quynh-Giao Steiner: New insights into dendritic cell biology and histiocytosis through a transgenic tumor model.

Antoine Tinel: Identification and characterization of a caspase-2 activiting complex.

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 2005

Christine Lavanchy, Technician
Estelle Säuberli, Technician
Luc A. Otten, Junior Faculty member
Teresa Bianchi, Postdoctoral fellow
Stanislav Rybtsov, Postdoctoral fellow
Quynh-Giao Steiner, Postdoctoral fellow
Stéphane Chevrier, Ph.D student
Céline Genton, Ph.D student
Julian Wagner, Ph.D student
Amine Salem, Trainee

Recent publications

Otten, L.A., Tacchini-Cottier, F., Lohoff, M., Annunziato, F., Scarpellino, L., Louis, J., Steimle, V., Reith, W., and Acha-Orbea, H. (2003).
Deregulated CIITA expression leads to a strong TH2 bias in CD4 T lymphocytes.
J. Immunol. 170, 1150-1157.

Finke, D., Luther, S.A., and Acha-Orbea, H. (2003).
The role of neutralizing antibodies for mouse mammary tumor virus transmission and mammary cancer development.
Proc. Natl. Acad. Sci. USA 100, 199-204.

Development of a model for human histiocytosis: transgenic mice developing dendritic cell tumors

Dendritic cells are the key regulators of the immune response. They control the induction of immune response and immune tolerance and also direct the type of immune response that will be induced after infection with different pathogens.

Topic

We have recently obtained a first mouse model for multi-system human histiocytosis. In this human disease, dendritic cells accumulate in different lymphoid and nonlymphoid organs and lead to severe morbidity and mortality. Little is known about the etiology of this disease, especially in the most severe multi-system disease involving bone, liver, skin and spleen. As a first approach we generated two transgenic mouse lines, which specifically express an oncogene in dendritic cells. These mice develop, depending on oncogene expression levels, dendritic cell tumors either quickly (after 4 months) or slowly (after 12 months). The transformed cells have maintained all the features of normal dendritic cells and show striking similarity to the severe forms of human disease. These mice allow on one side to propose mechanisms of disease inductions and new forms of treatment and etiology of human disease, on the other side represent long-sought tools to better understand the dendritic cell biology.

Current projects

In a first phase dendritic tumor cells are characterized and compared to freshly isolated dendritic cells. So far we found that these two types of dendritic cells show identical behaviors in antigen (cross)-presentation, cytokine production, activation and differentiation induced by different activation signals. We are currently using this model to address the genes involved in human disease as well as questions addressing dendritic cell differentiation and function.

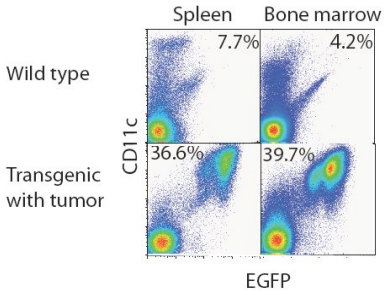


Figure 1: *Transgene expression (EGFP) is specific for dendritic cells (CD11c). Tumor cells are found in Bone marrow and spleen.*

Recent publications

J.C. Ramirez, D. Finke, M. Esteban, J.P. Kraehenbuhl, and H. Acha-Orbea. (2003). Nasal inoculation of modified vaccinia virus Ankara (MVA) is safe in mice. Arch. Virol. 148(5), 827-39.

Waldburger, J.M., Rossi, S., Hollander, G., Rodewald, H.R., Reith, W., and Acha-Orbea, H. (2003). Characterization of the CD4 T cell compartment in mice lacking the promotor IV of the class II transactivator gene. Blood 101, 3550-3559.

LeibundGut-Landmann, S., Waldburger, J.M., Reis e Sousa, C., Acha-Orbea, H., and Reith, W. (2004). MHC class II expression is regulated differentially between plasmacytoid and conventional dendritic cells. Nature Immunol. 5, 899-908,

Didierlaurent, A., Ramirez, J.C., Gherardi, M., Zimmerli, S.C., Graf, M., Acha-Orbea, H., Pantaleo, G., Wagner, R., Esteban, M., Kraehenbuhl, J.P., and Sirard, J.C. (2004). Attenuated poxviruses expressing a synthetic HIV protein stimulate HLA-A2-restricted cytotoxic T-cell responses. Vaccine 22, 3395-3403.

Ingold, K., Zumsteg, A., Tardivel, A., Huard, B., Steiner, Q.G., Cachero, T.G., Qiang, F., Gorelik, L., Kalled, S.L., Acha-Orbea, H., Rennert, P.D., Tschopp, J., and Schneider P. (2005). Identification of proteoglycans as the APRIL-specific binding partners. J. Exp. Med. 201, 1375-1383

Plasma cell differentiation during an immune response

During an immune response, B cells differentiate in a T cell-dependent fashion from naive B cells to memory and short- and long-lived plasma cells. B cells can either differentiate in germinal centers where they perform isotype switch and affinity maturation and become short- and long-lived plasma cells or alternatively in extrafollicular regions where they differentiate into short-lived isotype switched but not affinity-matured plasmablasts.

Topic

In collaboration with Marie and Bernard Malissen from Marseille, we characterize the B cell response in their knockin mice, which carry a LAT adaptor molecule with a point mutation in position 136. A Tyrosine, which after phosphorylation serves as a docking site for PLCγ is mutated into Phenylalanine. Therefore T cells cannot induce a calcium flux after activation. Nevertheless, after 3 weeks these mice show differentiation of $CD4^+$ T cells which have an effector TH2 phenotype. As a consequence B cells are activated and hyperimmunoglobulinemia IgG1 and IgE is observed.

Current projects

When analyzing these mice we surprisingly found, that all the B cell populations found in an antigen-induced immune response are found. Each B cell follicle is filled with large germinal centers and all the classical plasma and memory B cell populations are present. These mice show a polyclonal B cell activation without preference for autoreactive B cells. Nevertheless they develop antibody-dependent autoimmunity early in life.
The role of different surface molecules in T cell-B cell interaction can now be studied using genetic, adoptive transfer and *in vitro* approaches.

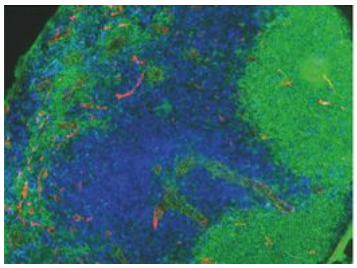


Figure 2: *Distribution of TZ cells (blue) B cells (green) and endothelia in a naive lymph node.*

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 Institute of Biochemistry in 2003.

Group members 2005

Simona Grossi, Postdoctoral fellow
Mathieu Delannoy, Ph.D student
Kerstin Gari, Ph.D student

Maintenance of genome integrity in human cells

The cellular response to DNA damages is an early defence mechanism against the genesis of tumours. What we currently know about the DNA damage response often derives from studies of cancer prone disorders. Many of the culprit genes encode proteins that either signal the presence of lesions in the DNA or directly repair chemically modified DNA or aberrant DNA structures. Inactivation of these caretaker genes precipitates the development of tumours.

Topic

Our current focus is the molecular function of proteins involved in Fanconi anemia. Fanconi anemia is a recessive disorder characterized by bone marrow failure, congenital abnormalities and early cancer predisposition. Recent developments suggest that FA proteins act within a network of proteins that function to counteract the deleterious consequences of lesions in the DNA during the process of DNA replication. FA cells exhibit high levels of spontaneous chromatid/chromosome breaks and radial chromosomes and are hypersensitive to a broad spectrum of genotoxic agents. Up to now, twelve FA complementation groups have been reported and eleven FA genes have been identified and cloned (*FANCA*, *B*, *C*, *D1*, *D2*, *E*, *F*, *G*, *J*, *L*, *M*). Eight FA gene products (FANCA,-B,-C,-E,-F,-G,-L,-M) form a complex in the nucleus. The integrity of the FA nuclear complex is necessary for the monoubiquitination of FANCD2 and for the targeting of Ub-FANCD2 to chromatin. Ub-FANCD2 accumulates within distinct nuclear foci, detected by immunofluorescence staining, which presumably reflect nuclear sites of ongoing DNA repair.

Current projects

We are using a combination of biochemical and cellular approaches to define the roles of FA proteins in DNA repair and DNA replication.
To define precisely in which context FA proteins are acting, we have studied the spatiotemporal organization of FANCD2 in replicating cells.

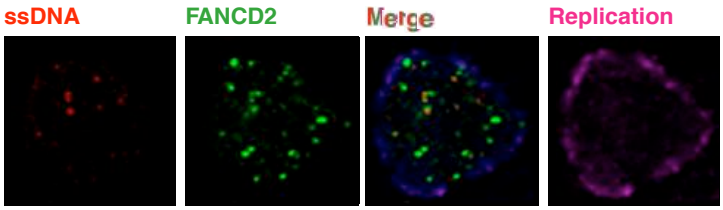


Figure 1: Dynamics of FANCD2 in S phase. *One representative nucleus is shown. Red staining: nuclear regions enriched in ssDNA intermediates. Green staining: FANCD2. Magenta staining: Replication factories in a mid S phase cell. DNA replication is confined to the nuclear periphery in mid S.*

FANCD2 is not recruited to replication factories but accumulates from mid S phase within sub-nuclear regions enriched in single-stranded DNA intermediates (Figure 1). The accumulation of single-stranded DNA is induced by DNA damaging agents and most likely arises as a consequence of replication catastrophes. These observations suggest that FANCD2 does not participate in the process of replication *per se* but acts on replicated chromatin to restore the integrity of damaged chromosomes.

Recent publications

Thorel, F., Constantinou, A., Dunand-Sauthier, I., Nouspikel, T., Lalle, P., Raams, A., Jaspers, N.G., Vermeulen, W., Shivji, M.K., Wood, R.D., and Clarkson, S.G. (2004).
Definition of a short region of XPG necessary for TFIIH interaction and stable recruitment to sites of UV damage.
Mol. Cell Biol. 24, 10670-10680.

Constantinou, A., and West, S.C. (2004).
Holliday junction branch migration and resolution assays.
Methods Mol. Biol. 262, 239-253.

Ciccia, A., Constantinou, A., and West, S.C. (2003).
Identification and characterization of the human mus81-eme1 endonuclease.
J. Biol. Chem. 278, 25172-25178.

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.

Lalle, P., Nouspikel, T., Constantinou, A., Thorel, F., and Clarkson, S.G. (2002).
The founding members of xeroderma pigmentosum group G produce XPG protein with severely impaired endonuclease activity.
J. Invest. Dermatol. 118, 344-351.

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.

We are combing DNA fibers to analyze the many parameters that determine DNA synthesis during S phase (Figure 2): a) the overall number of active replication origins; b) the firing of new origins of replication; c) the rate of movement of ongoing replication forks; d) and/or the occurrence of stalled replication forks. With this experimental approach, we will investigate the dynamics of DNA replication in FA-deficient cells and evaluate the impact of FA proteins on the DNA damage response in S phase.

Figure 2. DNA fiber analysis of DNA replication in human cells. *Cells were labelled sequentially with one pulse of iododeoxyuridine (IdU) followed by one pulse of chlorodeoxyuridine (CldU). DNA fibers were incubated with specific antibodies against the halogenated nucleotides (IdU labelled in red; CldU labelled in green). This figure shows examples of an ongoing replication fork (A), new initiation during the IdU pulse (B), new initiation during the CldU pulse (C), termination during the CldU pulse (D), termination or fork stalling during the IdU pulse (E).*

To define the biochemical properties of FA proteins, we are expressing and purifying tagged and untagged versions of FA proteins from insect cells. Some of these proteins can be purified in isolation whereas other factors are stable only when co-expressed with other subunits of the FA core complex. With pure proteins in hands, we are probing specific interactions between FA proteins and DNA substrates that mimic repair intermediates. Protein-DNA complexes are analyzed further by electron microscopy.

These cellular and biochemical studies will provide a conceptual framework for the development of cell free systems to dissect FA mediated processes. Cell free systems are amenable to fractionation, complementation and ultimately reconstitution with purified or partially purified repair factors, a prerequisite to our understanding of FA protein function in molecular details.

Our ambition is to contribute to our knowledge of the DNA damage response and provide opportunities to devise rational anti-cancer 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 on antibody-mediated targeting of antigenic MHC/viral peptide complexes on tumor cells.

MALARIA: search of 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.

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.

MALARIA: search of antigens for the development of protective vaccines

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.

The impossibility of culturing sporozoites outside the mosquitos renders this vaccination approach impracticable on a large scale. Thus, considerable effort has be devoted to the identification of highly antigenic sporozoite derived antigens which can substitute for the whole sporozoite

Group members 2005

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Patricia Ntsama, Trainee

Recent publications

Prato, S., Maxwell, T., Pinzon-Charry, A., Schmidt, C.W., Corradin, G. and Lopez, J.A. (2005).
MHC class I-restricted exogenous presentation of a synthetic 102-mer malaria vaccine polypeptide.
Eur. J. Immunol. 35, 681-689.

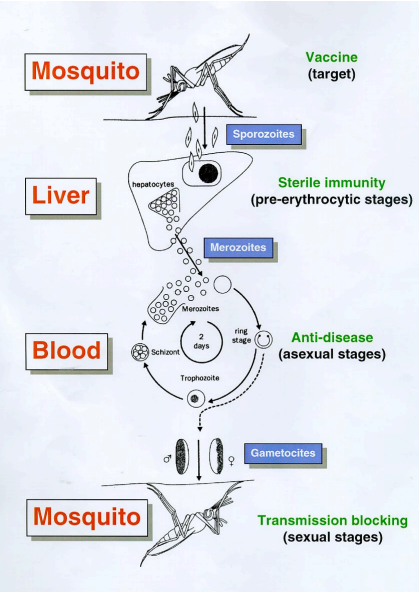
Singh, S., Soe, S., Roussilhon, C., Corradin, G. and Druilhe, P. (2005).
Plasmodium falciparum merozoite surface protein 6 displays multiple targets for naturally occurring antibodies that mediate monocyte-dependent parasite killing.
Infect. Immun. 73, 1235-1238.

Meraldi, V., Romero, J.F., Kensil, C. and Corradin, G. (2005).
A strong CD8+ T cell response is elicited using the synthetic polypeptide from the C-terminus of the circumsporozoite protein of Plasmodium berghei together with the adjuvant QS-21: quantitative and phenotypic comparison with the vaccine model of irradiated sporozoites.
Vaccine 23, 2801-2812.

Villard, V., Kalyuzhniy, O., Riccio, O., Potekhin, S., Melnik, T.N., Kajava, A.V., Ruegg, C. and Corradin, G. (2005).
Synthetic RGD-containing alpha-helical coiled coil peptides promote integrin-dependent cell adhesion.
J. Pept. Sci.

in the design of anti-malaria vaccines. A second target of vaccine intervention is represented by the erythrocytic stage of the parasite and its specific antigens.
Our approach to vaccine development involves the use of long polypeptides containing various B, T-helper, and T-cytotoxic epitopes. The use of longer antigen fragments will also provide epitopes capable of binding to the various MHC class I and II molecules, thus overcoming the MHC restriction observed in response to shorter epitopes. This approach has led to numerous malaria phase I clinical trials in record time (*P. falciparum* and *P. vivax* circumsporozoite protein, MSP-3, GLURP).
While this work continues in order to optimize the vaccine formulation to be used in further clinical trials, the availability of the *P. falciparum* genome provides an ideal basis from which to identify new proteins associated with protective immune responses.
To this effect, we performed a selection of new antigens using a genome-wide approach complemented with high-throughput peptide synthesis. We first identified potential protein antigens present on the surface of asexual malaria blood stages through bioinformatics and published transcriptome and proteome analysis. Amongst the proteins identified, we selected those that contain protein regions, which are relatively short (30-50 amino acid residues) and structurally stable as isolated fragments. 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 are immunogenic in mice. Further characterization of these proteins using mouse specific or affinity purified human antibodies is underway. In order to select the most appropriate protective antigens, a series of assays like antibody dependent cytotoxic inhibition (ADCI) and growth inhibition assay (GIA) are being employed to determine the biological properties of purified 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 of general application, and can be applied to any other pathogen where protection is mediated by antibodies.

Malaria cycle



Group members 2005

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Alena Donda, Senior researcher
Valérie Cesson, Ph.D student
Kathrin Stirnemann, Ph.D student

CANCER: antibody-mediated tumor targeting of MHC Class I and MHC-related molecules

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.

We, and others, have demonstrated the efficiency of such bifunctional conjugates consisting of anti-TAA antibodies and Class I molecules to

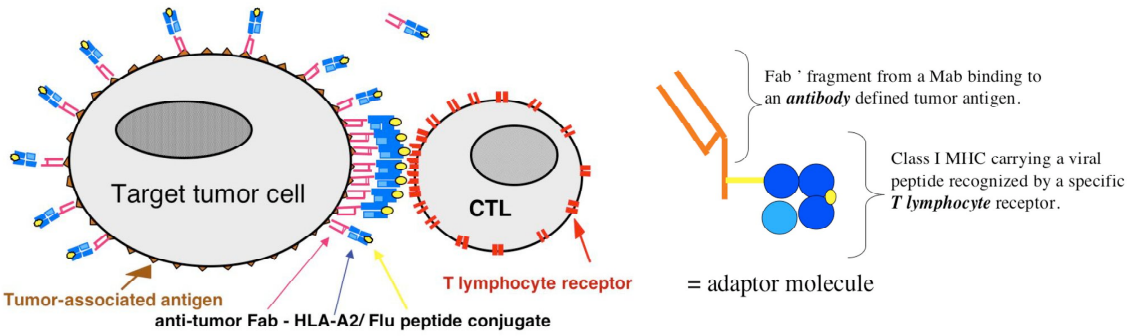

Figure 1: *Left part: schematic description of the binding to TAA and oligomerization of antibody Fab' fragment-MHC/peptide conjugates on the surface of a tumor cell, which induce the cooperative binding of several T-cells receptors leading to activation of the T effector lymphocyte, which will kill 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.*

Recent publications

Robert, B., Guillaume, P., Luescher, I., Doucey, M.-A., Cerottini, J.-C., Romero, P. and Mach, J.-P. (2001).
Redirecting anti-viral CTL against cancer cells by surface targeting of monomeric MHC class-I viral peptide conjugated to antibody fragments.
Cancer Immunity 1, 2
(http://www.cancerimmunity.org).

Donda, A., Cesson, V., Mach, J.P., Corradin, G., Primus, F.J. and Robert, B. (2003).
In vivo targeting of an anti-tumor antibody coupled to antigenic MHC class I complexes induces specific growth inhibition and regression of established syngeneic tumor grafts.
Cancer Immunity 3, 11
(http://www.cancerimmunity.org).

Buchegger, F., Adamer, F., Schaffland, A.O., Kosinski M., Grannavel, C., Dupertuis, Y.M., de Tribolet, N., Mach, J.P. and Delaloye, A.B. (2004).
Highly efficient DNA incorporation of intratumourally injected [125I]iododeoxyuridine under thymidine synthesis blocking in human glioblastoma xenografts.
Int. J. Cancer 110 (1), 145-9.

Germain, C., Larbouret, C., Cesson, V., Donda, A., Held, W., Mach, J.P., Pelegrin, A., Robert, B. (2005).
MHC class I-related chain a conjugated to antitumor antibodies can sensitize tumor cells to specific lysis by natural killer cells.
Clin. Cancer Res. 11 (20), 7516-22.

activate specific CTLs *in vitro* in the presence of different tumor cell lines expressing appropriate TAAs. We originally showed that even monomers of soluble class I/antigen chemically conjugated to anti-TAA Fab fragment efficiently induce T cell cytotoxicity only when oligomerized on the tumour cells (Figure 1) (Robert et al. 2001). Indeed, soluble conjugates in absence of tumor cells positives for the TAA, did not induce T cell activation, as shown by the absence of calcium release by specific T cells. This characteristic should increase the efficacy of the conjugate at the tumour site, while reducing side effects at the periphery.
Next, we have demonstrated in an entirely in vivo model of syngeneic colon carcinoma that this new tumor immunotherapy strategy can function in immunocompetent mice that are transgenic for a T cell receptor specifically directed against an antigenic peptide from the ovalbumin in the context of H-2K^b (Donda et al. 2003). We showed that conjugates of anti- tumor antibody-MHC class I, can provide tumor protection and tumor regression in an autologous environment. We are now testing such conjugates in 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 (Fig.2) (Cesson et al., in preparation).

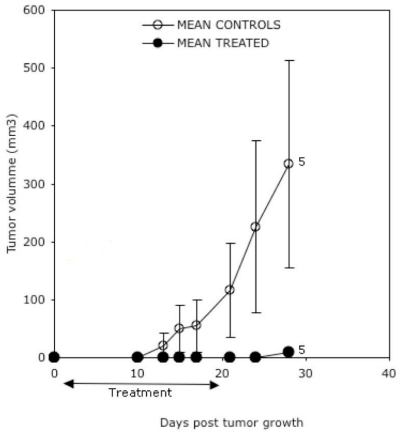

Figure 2: *Systemic injection of anti-CEA-H-2D^b/GP33^{XL} conjugate in LCMV-infected mice induces specific tumor growth inhibition of syngeneic subcutaneous carcinoma grafts. Treated mice received a total of 6 conjugate injections every two days starting one day after grafting. Only one out of 5 treated mice showed a tiny tumor, one week after treatment arrest, whereas all 7 control mice had fast growing tumors.*

Antibody-CD1d bifunctional molecules 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 and NK 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, coupled or genetically fused to the tumor targeting part, such as an antibody fragment with high 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.

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 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. Last and 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 already 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, in collaboration with H.R. MacDonald from the Ludwig Institute We are now developing *ex vivo* assays to test the fusion on mouse NKT cells from liver and spleen. The next step will be the *in vivo* testing of the CD1d fusion for its capacity to inhibit tumor growth in a lung metastasis model. For this purpose, the B16 melanoma cell line has been transfected with the human HER2 antigen and the Luciferase gene has been introduced by retroviral infection. This will allow the *in vivo* monitoring of the tumor growth and of the effect of treatment by bioluminescence imaging.

In another similar approach, our former coworker, B. Robert, has developed a conjugate between anti-TAA mAbs and the MIC-A molecule, which is the specific ligand for the NKG2D activating receptor from NK cells. His group, in collaboration with us and W. Held from the Ludwig Institute, has shown that anti-TAA-MIC-A conjugates coated on tumor cells can activate NK cells and induce them to kill the coated tumor cells (Germain et al. 2005). Thus, our general approach of antibody targeting of antigenic MHC complexes, or MHC-related molecules, on tumor cells represents promising novel strategies of cancer immunotherapy.

Gian-Paolo Dotto, Professor

G. 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.

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Control of epithelial stem cell potential versus differentiation and tumorigenesis

We are interested in molecular mechanisms that control self-renewal of epithelial cell stem cell populations versus their irreversible commitment to differentiation. Understanding the balance between epithelial self-renewal and differentiation is of great importance for normal organ morphogenesis and homeostasis. Additionally, transformation of epithelial cells is closely linked to disturbances of their normal growth/differentiation program. An elucidation of this program and its possible alterations are of great potential impact for new therapeutic approaches to human tumors, which are mostly of epithelial origin.
We use primary keratinocytes and skin as our main experimental model system, and are assessing how the regulatory mechanisms elucidated in this system apply to specific types of human epithelial tumors.
Primary keratinocytes provide an ideal experimental system to dissect the signaling pathways involved in epithelial growth/differentiation control. Relative to established cell lines, use of a primary system eliminates the possibility of alterations in critical regulatory pathways that may occur during prolonged propagation in culture. Primary keratinocytes are easily grown in large numbers, and their differentiation can be induced under well-defined culture conditions, with a rapid and homogeneous response amenable to careful biochemical analysis. Gene transfer technology (transient transfections, adenoviral and retroviral vectors) together with the use of keratinocytes derived from gene knockout and transgenic mice, make it possible to assess the specific contribution of individual genes to the control of the differentiation process. Finally, grafting of cultured keratinocytes together with other "instructing" cell types (dermal fibroblasts; dermal papilla cells) is sufficient to reproduce the complex differentiation program that occurs in vivo in the epidermis as well as hair follicles.
Within the proliferative compartment of the epidermis, at least two kinds of keratinocyte populations are thought to exist : totipotent "stem cells", with an indefinite self renewal potential and capable of generating all other types of growing and differentiating keratinocytes; and "transient amplifying cells", capable of a limited number of cell divisions and already committed towards differentiation. Primary keratinocyte cultures contain similarly distinct populations with different: i) integrin expression levels and/or adhesive properties; ii) clonogenic growth potential; iii) ability to differentiate along distinct lineages of both interfollicular epidermis and hair follicles. Additionally, keratinocyte populations that have exited the cell cycle can be distinguished between reversibly and irreversibly committed to differentiation.

Our main working hypotheses, depicted in the diagram below, is that there is a dynamic equilibrium between keratinocyte stem cells, transit amplifying populations and cells that are reversibly versus irreversibly committed to differentiation. According to this model, the size of stem cell populations is likely to be influenced by the number of surrounding transit amplifying cells that are in turn in equilibrium with cells that have withdrawn reversibly versus irreversibly from the cell cycle. It is therefore likely that the number of stem cells can be controlled through mechanisms acting at multiple levels, including relative late steps in the sequence of events leading to keratinocyte maturation.

Recent publications

Okuyama, R., Lefort, K. and Dotto, G.P. (2004).
A dynamic model of keratinocyte stem cell renewal and differentiation : role of the p21WAF1/Cip1 and Notch1 signaling pathways.
J. Invest. Dermatol. 9, 248-52.

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, 551-62.

Mammucari, C., Tommasi di Vignano, A., Sharov, A.A., Havrda, M.C., Roop, D.R., Botchkarev, V.A., Crabtree, G.R. and Dotto, G.P. (2005).
Integration of Notch1 and Calcineurin/NFAT signaling pathways in keratinocyte growth and differentiation control.
Dev. Cell 8, 665-76.

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.

Grossi, M., Hiou-Feige, A., Tommasi Di Vignano, A., Calautti, E., Ostano, P., Lee, S., Chiorino, G. and Dotto, G.P. (2005)
Negative control of keratinocyte differentiation by Rho/CRIK signaling coupled with up-regulation of KyoT1/2 (FHL1) expression.
Proc. Natl Acad. Sci. USA 102, 11313-11318.

Wang, J., Devgan, V., Corrado, M., Prabhu, N.S., El-Deiry, W.S., Riccardi, R., Pandolfi, P.P., Missero, C.G. and Dotto, G.P. (2005).
GITR is a p21 Cip1/WAF1 transcriptional target conferring resistance of keratinocytes to UV-induced apoptosis.
J. Biol. Chem. 280, 37725-37731.

Lefort, K. and Dotto, G.P. (2004).
Notch signaling in the integrated control of keratinocyte growth/differentiation and tumor suppression.
Seminars Cancer Biol. 14, 374-386.

Dotto, G.P. and Silke, J. (2004).
More than cell death: caspases and caspase inhibitors on the move.
Dev. Cell 7, 2-3.

Dotto, G.P. and Cotsarelis, G. (2005).
Rac1 Up for Epidermal Stem Cells.
Science 309, 890-891.

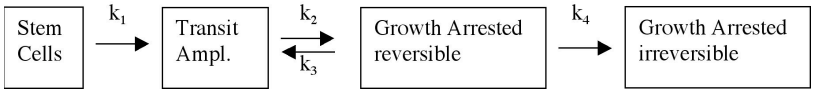

Figure 1: *Our working model of a dynamic equilibrium between keratinocyte populations with different commitment to differentiation.*

We are engaged in two main lines of research:

Basic control of keratinocyte growth and differentiation

Within the conceptual framework illustrated above, we will continue to explore basic mechanisms that control the balance between keratinocyte growth and differentiation at three distinct levels:

1) Control of the transition between keratinocyte stem cell populations and transit amplifying cells. Like in other organ systems, keratinocyte stem cells are only a small fraction of cells in the epidermis, with no unequivocal biochemical markers, so that their analysis is mostly limited to functional rather than biochemical assays. A possible role of developmental signals in control of epithelial stem cell populations is an important but still largely undefined area. Notch signaling provides a highly conserved mechanism for control of cell fate determination and differentiation in a wide variety of developmental and non-developmental processes. We will be examining the role that this pathway plays in promoting the transition of keratinocyte stem cell populations into the transit amplifying compartment. More mechanistically, in mouse primary keratinocytes, we have established that the cell cycle inhibitor $p21^{WAF1/Cip1}$ is a direct target of Notch1 signaling and that, besides the cell cycle, both Notch1 and $p21^{WAF1/Cip1}$ play a critical role in control of keratinocyte stem cell potential. Global analysis of gene expression has revealed several important transcriptional targets of Notch1 and p21 function in keratinocytes, and we will be analyzing these genes for their function in control of keratinocyte stem cell renewal versus commitment to differentiation.

2) Control of the transition between growing transit amplifying keratinocyte populations and reversibly growth arrested cells. The vast majority of growing keratinocytes in culture, as well as in the basal layer of the epidermis *in vivo,* is represented by transit amplifying cells. Therefore, a detailed biochemical and genetic analysis of the signaling mechanisms that control the exit of transit amplifying cells from the cell cycle and entry into differentiation can be readily performed. More specifically, we will be examining the functional integration of signals that control keratinocyte cell/cell adhesion, growth and differention, with particular emphasis on E-cadherin adhesion complexes, Src/Fyn tyrosine kinases and small GTPases of the Rho family. We have recently shown that increased activity of the Rho GTPase at early times of keratinocyte differentiation leads to Fyn tyrosine kinase activation which in turn results in ß- and γ-catenin tyrosine phosphorylation and stabilization of E-cadherin adhesion complexes. Separately from cell/cell adhesion, we have found that the Rho GTPase plays an important role in control of keratinocyte differentiation and gene transcription, and we will be analyzing the transcriptional targets of Rho in the keratinocyte cell type.

3) Control of the irreversible phase of keratinocyte differentiation. The irreversible phase of keratinocyte differentiation can be considered as a specialized form of programmed cell death, without classical features of apoptosis (such as DNA fragmentation and cell membrane modifications) but with possibly overlapping regulatory pathways. We have found that $p21^{WAF1/Cip1}$ – and probably Notch1 - , besides their involvement in control of stem cell potential, play an important function in determining the irreversible commitment of keratinocyte populations to differentiation.

Among the p21 and Notch1-regulated genes we have found a number of genes connected with TNF-α signaling and/or downstream death triggering pathways. We will be exploring the contribution of these genes to the irreversibility of the terminal differentiated state.

Control of epithelial tumor development

Keratinocyte stem cell populations are the likely targets of chemically- or *ras*- induced mouse skin carcinogenesis. We have previously shown that loss of $p21^{WAF1/Cip1}$, in parallel with an increased fraction of keratinocyte stem cells, results in increased susceptibility to skin tumor development and it is likely that similar consequences may ensue from loss of Notch1. Like in the mouse system, Notch1 activation has been reported to promote the commitment of human keratinocyte stem cell populations towards differentiation. However, in contrast to the mouse cells, we have found that increased Notch1 activity in primary human keratinocytes does not result in induction of $p21^{WAF1/Cip1}$ expression or immediate growth arrest. Rather, increased Notch1 activation causes drastic growth suppression of tumor cells derived from cervical carcinomas, a major type of human keratinocyte-derived tumor. Besides cervical carcinoma cells, other types of human cancer cells, most notably prostate carcinomas and small cell lung carcinomas, have been reported to be growth suppressed by increased Notch1 activity. We are therefore examining the mechanisms underlying Notch1-mediated tumor suppression, to determine to which extent such mechanisms overlap with the ones that promote, in normal cells, the exit from the stem cell compartment and entry into differentiation.

Nicolas Fasel, Associate Professor

Nicolas Fasel is an associate professor at the Medicine Faculty 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 independent 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 2003, he is co-director of the Department.

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Iveth Gonzalez, Ph.D student
Annette Ives, Ph.D student
Hugues Jaccard, Ph.D student
Béatrice Cuche, Trainee

Structural and 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, 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 events underlying these developmental changes in the parasite 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 the *Leishmania* pathogenicity was aimed mainly at the characterization of the functional role of histone H1 and its importance in parasite survival (Part A), on the cell death process in Leishmania (Part B) and finally on the identification of virulent markers in New World *Leishmania* of the *Viannia* subgenus (Part C).

(A) The role of histone H1 in chromatin condensation and infectivity of Leishmania parasites

In *Leishmania*, the level of histone H1 expression is tightly regulated and is augmented in the infectious stages of the parasite life cycle. In the last years, we have focused our research on analysing the expression of histone H1 in *Leishmania*. The deduced amino acid sequence of *Leishmania major* histone H1 cDNA revealed the presence of characteristic histone H1 amino acid motifs. However, the open reading frame was of an unusually small size for histone H1 (105 amino acids) because it lacked the coding potential for the central hydrophobic globular domain of linker histones present in other eukaryotes. We provided biochemical and immunocytochemical evidence to prove that the H1 protein was indeed a *L. major* nuclear histone H1, and that it was differentially expressed during the life cycle of the parasite. Together, these observations suggested that the different levels of this histone H1 protein could influence the varying degrees of chromatin condensation during the life cycle of the parasite. This expression parallels an increase in DNAse enzymatic activity and oligosomal DNA fragmentation. Structurally, the *Leishmania* histone H1 resembles the C-terminal domain present in histone H1 from higher eukaryotes which was shown to be important for chromatin binding and in the activation of the apoptotic DNA fragmentation factor DFF40/CAD. Recently, we investigated the functional role of the *Leishmania* histone H1 and demonstrated its importance in chromatin condensation and parasite infectivity. Addition of exogenous full-length histone H1 showed a strong effect on micrococcal digestion of parasite nuclei and acted as a signal for death as evidenced by phosphatidylserine exposure and nuclear chromatin condensation.

Increasing H1 expression in parasites diminished their virulence, thus demonstrating that histone H1 modulates parasite infectivity and must be tightly regulated to avoid uncontrolled death of the parasites.

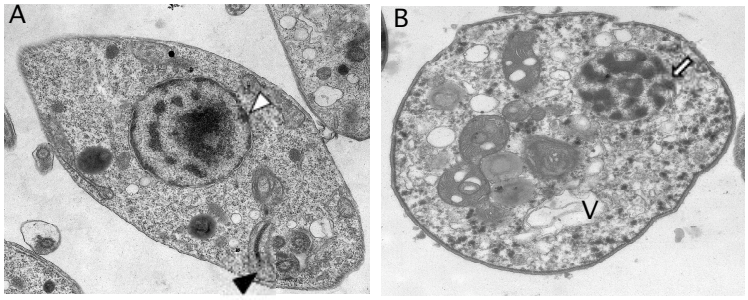

Figure 1: *Electron microscopy analysis of Leishmania parasites transduced with histone H1 peptide. Log phase L. major promastigotes were incubated in the absence (A), or presence of H1(B) and monitored for changes in cell morphology. White arrowhead intact nuclei; black arrowhead, kinetoplast; V, vacuole; white arrow, condensed nuclei. Parasites treated with H1 showed apoptotic cell death features including condensed nuclear chromatin, disruption and breakdown of organelles, a vacuolarised cytoplasm and a more rounded appearance when compared with control parasites. Magnification 8900x.*

(B) Cell death in the intracellular stage of *Leishmania*

In the course of our studies on the expression of histone H1 and differential chromatin condensation during the life cycle of the parasite, we observed a DNA fragmentation pattern at specific stages of the parasite life cycle. This was reminiscent of cells undergoing apoptosis. The DNA fragmentation which was observed, without induction, in the infectious stages of the parasite (metacyclics and amastigotes), correlated with the presence of internucleosomal nuclease activity, visualisation of 45 to 59 kDa nucleases and detection of TUNEL-positive nuclei. Our recent work demonstrated that under a variety of stress conditions such as serum deprivation, heat shock and nitric oxide, cell death could be induced leading to genomic DNA fragmentation into oligonucleosomes. The DNA fragmentation was not dependent on active effector downstream caspases nor on the lysosomal cathepsin L-like enyzmes CPA and CPB. These data are consistent with the presence of a caspase-independent cell death mechanism in *Leishmania,* induced by stress and differentiation that differs significantly from metazoa.

A conserved family of caspase-related proteases, namely the metacaspases, have been found in plants, fungi, yeast and protozoa. Their significant sequence and structural similarities with caspases suggest that metacaspases could play a similar role to caspases in programmed cell death (PCD). The role of *Saccharomyces cerevisiae* metacaspase (YCA1) in yeast PCD has been demonstrated. Similarly, overexpression of one of the five *Trypanosoma* metacaspases, TbMCA4, and the *Arabidopsis* metacaspases AtMCP1b and AtMCP2b significantly promoted PCD in yeast. A single gene encoding a putative metacaspase (LmjMCA) is present in the genome of *Leishmania major*, however it is not known if it is involved in PCD. In order to define its functional role, we have cloned the LmjMCA and evaluated its activation and effect when overexpressed in yeast. An YCA1 disrupted *S. cerevisiae* strain was complemented with the LmjMCA. Cells were induced for protein production and exposed to H_2O_2 as a PCD inducer. Our results demonstrated that LmjMCA plays a similar role to YCA1 in yeast PCD. In parallel, we identified a substrate specific for Leishmania metacaspase. These results will allow the evaluation of the role of metacaspase in trypanosomatid PCD.

(C) Identification of virulent markers in Leishmania Viannia species

In collaboration with Prof. Nancy Saravia and Dr. John Walker, CIDEIM, Cali Colombia.

Mucocutaneous leishmaniasis (MCL) caused by *Leishmania* of the *Viannia (V.)* subgenus is one of the most severe forms of human tegumentary leishmaniasis. It produces a wide variety of symptoms, such as nasal congestion, deformities, malnutrition, respiratory obstruction, and

Recent publications

Acestor, N., Masina, S., Walker, J., Saravia, N.G., Fasel, N., and Quadroni, M. (2002).
Establishing two-dimensional (2-DE) gels for the analysis of *Leishmania* proteomes.
Proteomics 2, 877-79.

Zangger, H., Mottram, J.C., and Fasel, N. (2002).
Caspase–independent cell death in *Leishmania* induced by stress and differentiation: Programmed cell death or necrosis.
Cell Death and Differentiation 9, 1126-1139.

Masina, S., Gicheru, M., Demotz, S., and Fasel, N. (2003).
Protection against cutaneous leishmaniasis in outbred vervet monkeys with a recombinant Histone H1 antigen
J. Inf. Diseases 188, 1250-1257.

Monnerat, S., Martinez-Calvillo, S., Worthey, E., Myler, P.J., Stuart, K.D., and Fasel, N. (2004).
Genomic organization and gene expression in a chromosomal region of *Leishmania major*.
Mol. Bioch. Parasitology 134, 233-243.

Nakhaee, A., Taheri, T., Taghikhami, M. Mohebali, M., Salmanian, A.H., Fasel, N., and Rafati, S. (2004).
Antibody responsiveness against Type I cysteine protease of Leishmania Infantum is higher in asymtomatic dogs than in symptomatic dogs selected from a naturally infected population.
Vet Parasitol. 119, 107-23.

Rafati, S., Salmanian, A., Syed-Eslami, S., Masina, S., Schaff, C., and Fasel, N. (2004).
The *Leishmania major* Type I signal peptidase is a target of the immune response in humans.
Mol. Biochem. Parasitol. 135, 13-20.

Perrenoud, G., Messerli, F., Cousin, P., Beltraminelli, N., Thierry, A.C., Fasel, N., Demotz, S., Duchosal, M., and Moulon, C. (2004).
A recombinant rubella virus E1 glycoprotein as a rubella vaccine candidate
Vaccine 23, 480-488.

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.

in some cases leads to death. This disease has been a challenge for clinicians and researchers due to its uncommon occurrence and poorly understood pathogenesis. In MCL, after apparent cure of the primary lesion, secondary lesions may appear in the nasopharyngeal tissues of the infected host due to dissemination of the infection from the inoculation. Both nasal and oral lesions are characterized by a severe inflammatory reaction. These sites are evidently colonized by the dissemination of infected macrophages via haematogeneous and lymphatic routes, in humans as well as in the golden hamster model. New lesions can appear several years after healing of the original cutaneous lesion and may be triggered by trauma or inflammatory stimuli. Dissemination and the consequent metastatic lesions lead to the disfiguring morbidity associated with MCL. The spectrum of the biological outcome of infection is influenced not only by the host immune status but also by differences in parasite virulence.

Previous work established heterogeneity among strains of *L.* (*V.*) *panamensis* and *L.* (*V.*) *guyanensis* with respect to their capacities to disseminate and generate secondary metastatic lesions in the golden hamster model. Although all the derived clones induced primary cutaneous lesions, some disseminated from the initial lesion and exhibited different phenotypes in hamsters, allowing the classification of clones into two groups: metastatic (M+), and non-metastatic (M-).

Recently, we used (M+) and (M-) clones (together with clinical isolates of *L. Viannia* from mucocutaneous or cutaneous lesions) as tools for the identification of gene products associated with metastasis in New World *Leishmania* species. Comparative proteome analyses via 2D-electrophoresis (2-DE) coupled with peptide fingerprinting, mass spectrometry (MS) and bioinformatics enabled the identification of differentially expressed proteins including the enzyme tryparedoxin peroxidase (TPXN). The association between phenotype and differential expression of this protein was conserved in some, but not all *L. Viannia* isolates from mucosal or cutaneous lesions of patients with MCL or cutaneous leishmaniasis.

Infected macrophages are likely to be involved in metastasis and pathogenesis of nasopharyngeal lesions since tissue macrophages are the principal host cells of *Leishmania.* TPXN could have an important role in the initial infective step and in the metastatic process of *L. guyanensis*.

In our study, we analyzed the resistance to reactive oxygen species of *L. guyanensis* clones and *L. panamensis* strains presenting disparate metastatic phenotypes. We characterized TPXN at the DNA, protein and enzymatic activity level since this enzyme was shown to be present in different isoforms in (M+) and (M-) clones and strains. A specific isoform might be associated with increased resistance to reactive oxygen species.

Sanjiv Luther, Assistant Professor

Sanjiv Luther studied cell biology at the ETH in Zürich. 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. His present research focuses on the signals and cells that induce and define the T zone microenvironment in secondary lymphoid tissues.

Group members 2005

Stéphanie Zutter, Technician
Mirjam Britschgi, Ph.D student
Alexander Link, Ph.D student
Tobias Vogt, Ph.D student

Development and function of stromal cells in secondary lymphoid tissues

Secondary lymphoid tissues, such as lymph nodes and spleen, are the only sites where lymphocytes are efficiently activated during primary immune responses. While we have made considerable progress in understanding the biology of lymphocytes that recirculate through these organs, we know very little about the resident cells that form the 'niches' within this unique microenvironment. It has been recently discovered that stromal cell networks within these secondary lymphoid tissues not only serve as support structure, but actively control lymphocyte and dendritic cell migration and positioning by secreting chemotactic factors.
Our recent work has shown that stromal cells found within the T cell rich zone are an important source of the chemoattractants SLC and ELC responsible for bringing together antigen-presenting dendritic cells and recirculating T cells. Given the critical importance of this process for the initiation of adaptive immune responses, the interest of the lab is focused on further dissecting the development, phenotype and function of these poorly characterized T zone stromal cells.

Structure and function of secondary lymphoid tissues

Secondary lymphoid tissues are localized at strategic sites of the body to filter various fluids and to present these filtered antigens to recirculating lymphocytes. To perform this function these tissues are highly structured and can be divided into a B cell rich (B zone) and a T cell rich zone (T zone). The B zone consists mainly of a network of stromal cells that include follicular dendritic cells, and naive recirculating B cells. The T zone consists of a network of distinct stromal cells, mature dendritic cells and recirculating T cells of both the helper and cytotoxic subset. It is within the T zone where thousands of recirculating T cells enter through high endothelial venules and scan the microenvironment for the antigen they are specific for. This antigen is usually presented within the T zone by dendritic cells that have captured foreign antigen in the periphery and migrated into these tissues to encounter the rare T cell specific for the antigen. Interestingly, it is the resident T zone stroma that brings these two cell types together within the T zone by secreting the chemoattractants ELC and SLC.
Secondary lymphoid tissues have also been shown to be important for peripheral T cell homeostasis. T helper cells receive survival signals during their passage through the T zone when they recognize MHC class II molecules and cytokines such as IL-7. However, the cellular source of these molecules remains controversial.

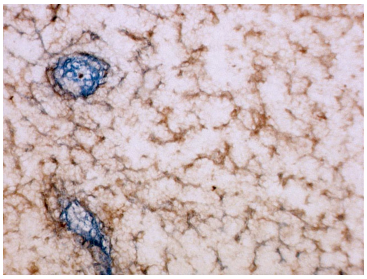

Figure 1: *T zone stromal cells form a dense network of fibroblast like cells throughout the T zone of secondary lymphoid tissues. gp38+ T zone stromal cells are depicted in brown, sites of lymphocyte entry (PNAd+ high endothelial venules) in blue.*

T zone stromal cells as source of chemoattractants

T zone stromal cells have been mainly characterized morphologically. They display fibroblast like morphology, associate with collagen fibres and can be selectively stained using the marker gp38 (Fig.1). Because of

Recent publications

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.

Luther, S.A., Ansel, K.M., and Cyster, J.G. (2003).
Overlapping function of CXCR5, IL-7 receptor a and CCR7 in lymph node development.
J. Exp. Med. 197, 1191-1198.

Finke, D., Luther, S.A., and Acha-Orbea, H. (2003).
The role of neutralizing antibodies for mouse mammary tumor virus transmission and mammary cancer development.
Proc. Natl. Acad. Sci. USA 100, 199-204.

Luther, S.A., Bidgol, A, Hargreaves, D., Schmidt, A., Matloubian, M., and Cyster, J.G. (2002).
Differing activities of CCL19, CCL21 and CXCL12 in lymphocyte and dendritic cell recruitment and lymphoid neogenesis.
J. Immunol. 169, 424-433.

Luther, S.A., and Cyster, J.G. (2001).
Chemokines as regulators of T cell differentiation.
Nature Immunol. 2, 102-107.

Luther, S.A., Lopez, T., Bai, W., Hanahan, D., and Cyster, J.G. (2000).
BLC expression in pancreatic islets causes B cell recruitment and lymphotoxin-dependent lymphoid neogenesis.
Immunity 12, 471-481.

their fixed location, T zone stromal cells were proposed to form migrational corridors along which lymphocytes travel. A more active function was suggested by our finding that T zone stromal cells are the major constitutive source of the chemokines ELC (CCL19) and SLC (CCL21) that promote the attraction of CCR7-bearing dendritic cells and T cells (Fig.2). Both the development and function of T zone stromal cells is strongly dependent on the membrane-bound cytokine lymphotoxin $\alpha\beta$ and to a lesser extent on the cytokine TNFα.
The importance of these stromal cells and the CCR7 ligands secreted by them is best illustrated in a natural mouse mutant (*plt*/*plt*) that we and others have shown to lack expression of both CCR7 ligands within lymph nodes and spleen. In *plt*/*plt* mice CCR7-expressing dendritic cells and T cells fail to accumulate in the T zone leading to an inefficient induction of T cell activation. At present it is unclear, what roles ELC and SLC play within lymphoid tissues. To start addressing this question, we have used transgenic mouse technology to either overexpress SLC or ELC in vivo, or to delete ELC (Luther and Cyster, unpublished) using gene targeting.

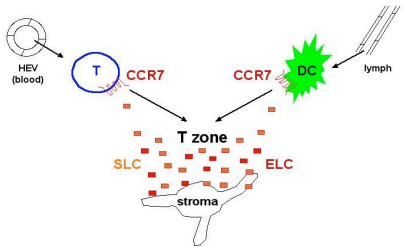

Figure 2: *Stromal cells resident in the T zone produce large amounts of the two CCR7 ligands, SLC and ELC. CCR7-expressing cells, such as naïve T cells entering from the blood stream and mature dendritic cells (DC) entering from the lymph respond to these chemoattractants by migrating into the T zone allowing their physical interaction.*

T cell stromal cells as source of survival and growth factors

Stromal cells in the bone marrow, thymus and B zone of secondary lymphoid tissues 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. Therefore, we propose that resident T zone stromal cells are ideally positioned not only to attract T cells and dendritic cells into their proximity but also to influence their survival, interaction, proliferation and differentiation.

To address these issues, we are currently

1. characterizing the respective roles of SLC and ELC in lymphoid tissue development and function using ELC knockout and plt/plt mice
2. studying the phenotype and development of ELC/SLC-expressing stromal cells within the T zone of spleen and lymph nodes
3. characterizing factors produced by ELC/SLC-expressing stromal cells that are involved in shaping dendritic cell and T cell survival, migration and interaction.

Perspective

Our present and future studies aim at improving our understanding of the T zone stromal cells responsible for generating the unique microenvironment where antigen-specific T cells are induced to proliferate and differentiate. These studies should help in designing intervention strategies to enhance or suppress immune responses within secondary lymphoid tissues and at sites of inflammation or cancer where similar stromal cell networks have been observed.

Andreas Mayer, Professor

Andreas Mayer studied chemistry and biology 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 2004 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.

Group members 2005

Véronique Comte-Miserez, Technician
Monique Reinhardt, Technician
Andrea Schmidt, Technician
Christopher Peters, Junior Faculty member
Tonie Baars, Ph.D student
Priscilla Brunetto, Ph.D student
Rosie Dawaliby, Ph.D student
Lydie Michaillat, Ph.D student
Heinz Neumann, Ph.D student
Christoph Reese, Ph.D student
Andreas Uttenweiler, Ph.D student
Anja Apel, Ph.D student
Marta Hoffman, Student

Membrane fusion and vesicle formation

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. We have addressed the issue using cell-free reconstitution of the fusion of yeast lysosomes (vacuoles). Factors involved in fusion have been characterized by kinetic analyses, genetics, pharmacological screens, biochemical fractionation, and by cytology in vivo and in vitro. Over the last two years we have made three major discoveries: First, a novel mechanism coordinating the machineries of membrane fusion and of membrane fission. Second, we have identified a signaling pathway mediating nutrient control over the membrane fusion/fission balance on yeast lysosomes. These results yield important novel insights into the regulation of the eukaryotic membrane fusion machinery. Finally, we have discovered and characterized the first hemifusion intermediate of a SNARE-dependent physiological fusion reaction. This hemifusion intermediate shows similar properties as in the fusion of viruses or of artificial liposomes suggesting that there may be a universal reaction pathway of membrane fusion in all of these systems. Thus, important findings were made that challenge and extend current pardigms on membrane fusion.
In a second project we have studied microautophagocytosis, an aspect of the controlled self-digestion of cytoplasmic compounds in lysosomes. We have identified several factors involved in this process and identified novel roles of specific lipids in microautophagic lysosome invagination. These studies have been pivotal in the characterization of microautophagy which is an essentially uncharacterized phenomenon – though it is induced in all eukaryotic cells under conditions of nutrient limitation or stress.

SNARE-dependent intracellular membrane fusion

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 on all compartments of the secretory and endocytic pathway share conserved factors and follow similar reaction pathways.
Fusion requires SNAREs which occur in compartment-specific cognate combinations of vesicular (v-) and target (t-) SNAREs. v- and t-SNAREs can form trans-complexes between the two fusion partners which may hold the membranes in close apposition. Little has been known about how this state of membrane docking evolves into lipid flux and fusion pore opening and how the fusion machine is connected to signal transduction cascades regulating membrane traffic in response to external stimuli. We address both issues in the cell free fusion of yeast vacuoles - a biological membrane system carrying all physiological components yet being very amenable to a multitude of experimental approaches. Since vacuole fusion shares many conserved features with fusion reactions on other compartments of other cell types it is a valid model for studying the general mechanism of membrane fusion.

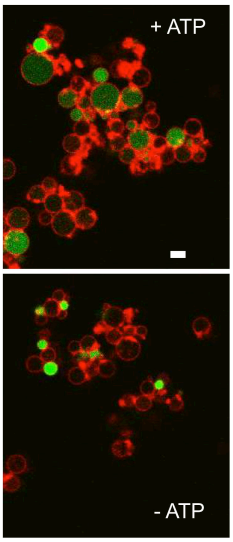

Figure 1: *Fusion of yeast vacuoles in vitro: vacuolar membranes are stained in red, the lumen of a fraction of the vacuoles is filled with the soluble fluorophore calcein. Fusion occurs only in the presence of ATP.*

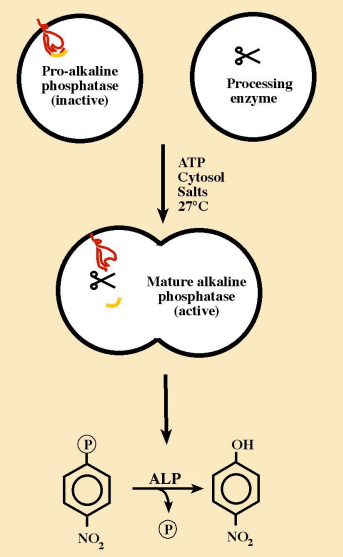

Figure 2*: Biochemical assay of vacuolar fusion activity in vitro. Vacuoles are separately prepared from strains expressing pro-alkaline phosphatase or the appropriate maturation enzyme. After mixing these populations fusion grants access of the maturase to the pro-enzyme, leading to mature, active alkaline phosphatase. Alkaline phosphatase serves as a simple coupled assay for fusion activity.*

Assay of lipid transition and the opening of fusion pores

Analysis of membrane fusion requires the ability to dissect fusion of the outer and inner lipid leaflets and the opening of a fusion pore. If combined with defined manipulations of membrane fusion proteins this should allow far-reaching conclusions. We have developed assays for lipid flow and for opening of small fusion pores which allowed us to define the first hemifusion intermediate for a SNARE-dependent fusion reaction (Reese et al., 2005). It shows similar properties as hemifusion intermediates in viral fusion. It precedes opening of the fusion pore which is the last and rate-limiting step (Reese and Mayer, 2005). Thus, extracellular (viral) and intracellular (SNARE-dependent) fusion may proceed via similar basic reaction pathways. We could demonstrate that hemifusion is preceded by trans-SNARE pairing and separated from this event by several molecular steps, supporting the notion that – in contrast to the widely held view - SNAREs dock membranes but do not suffice to fuse them.

How are membrane fission and fusion events coordinated?

Membrane traffic between the compartments of eukaryotic cells consists of cycles of membrane fission (vesicle formation) and membrane fusion (vesicle consumption). Fission and fusion must be balanced to guarantee membrane homeostasis - and they are intrinsically antagonistic reactions. Activated fusion machinery might immediately reverse membrane fission of a nascent vesicle, and vice versa, resulting in futile cycles of fission and fusion. We have recently identified functional and molecular interactions between proteins acting in fusion and fission (Peters et al., 2004). Dynamins promote membrane fission by GTP-driven changes of conformation and polymerization state while SNAREs dock membranes for subsequent fusion by forming complexes between t- and v-SNAREs from apposed vesicles. We discovered that the dynamin-like GTPase Vps1p, which is required for membrane fission, also functions in fusion of yeast vacuoles. The SNARE-activating ATPase Sec18p/NSF and the t-SNARE depolymerize the dynamin, thereby inactivating its fission activity. Aside from inactivating the fission activity of Vps1p, Vps1p depolymerization liberates the t-SNAREs for initiating docking and fusion. These results provide the first example suggesting that reciprocal control between fusion and fission components exists which may prevent futile cycles of fission and fusion. This aspect is of fundamental importance for the regulation of intracellular membrane traffic.

Connection of the fusion machine to signal transduction cascades

Organellar structure and dynamics are influenced by environmental conditions or by the cell cycle. The underlying regulatory events are largely unexplored. We have discovered a novel pathway regulating the balance of fusion and fission on yeast vacuoles/lysosomes. It requires the cyclin-dependent kinase Pho85p (a Cdk5 homolog) and the cyclin Pho80p. Pho85p signals the release of Vps1p via a cascade comprising protein phosphatase 1 (Glc7p) and its cofactor, the p47 homologue Shp1p. Under high phosphate conditions the Pho80p/Pho85p complex phosphorylates Shp1p and thereby activates the protein phosphatase 1 Glc7p. This releases Vps1p from the t-SNARE Vam3p and liberates the it to initiate downstream events of fusion. This establishes the first connection of the vacuolar fusion machinery to nutrient signaling. Future analyses will have to show whether a similar regulatory mechanism operates also in mammalian cells, in which the membrane association of dynamin is controlled by the Pho85 homologue Cdk5.

Recent publications

Kunz, J.B., Schwarz, H., and Mayer, A. (2004)
Determination of four sequential stages during microautophagy in vitro.
J Biol Chem. 279, 9987-96.

Peters, C., Bühler, S., Baars, T., and Mayer, A. (2004)
Mutual control of membrane fission and fusion proteins .
Cell 119, 667-678.

Reese, C., Heise, F., and Mayer, A. (2005)
Trans-SNARE pairing can precede a hemifusion intermediate in intracellular membrane fusion.
Nature 436, 410-414.

Uttenweiler, A., Schwarz, H., and Mayer, A. (2005)
Microautophagic vacuole invagination requires calmodulin in a Ca^{2+} independent fuction.
J. Biol. Chem. 280, 33289-97.

Reese, C., and Mayer, A. (2005) Transition from hemifusion to pore opening is rate-limiting for vacuole membrane fusion.
J. Cell Biol., in press.

Relevance for other systems

Several factors and events defined by our work on vacuoles also have roles in other fusion reactions, suggesting that they have broad relevance: Roles for Ca^{2+} release from the organellar lumen and for calmodulin have also been identified for fusion at the Golgi, at mammalian endosomes and at lysosomes. Protein phosphatase I controls also fusion at the Golgi and the VTC proteins may also function in fusion of ER-vesicles at the Golgi. Finally, the V_0 sector of the V-type H^+-ATPase, which is necessary to induce vacuolar hemifusion, is also required for regulated exocytosis of docked synaptic vesicles. Studying vacuole fusion hence elucidates general aspects of membrane traffic also relevant to vesicular transport in higher eukaryotic cells.

Microautophagic formation of intravacuolar vesicles

A second area of interest concerns dynamic changes of membrane shape, exemplified by the invagination of the vacuolar membrane during microautophagocytosis. Eukaryotic cells respond to stress or nutrient limitation by autophagocytosis, i.e. by the transfer of cytoplasm into lysosomes. This leads to controlled self-digestion and recycling of cellular compounds which enables the cell to adapt to the new requirements and to survive. Macroautophagy leads to the formation of double-layered vesicles out of membrane cisternae. Fusion of their outer membrane with lysosomes transfers the inner membrane and its cytoplasmic content into lysosomes for degradation. Microautophagy occurs through direct invagination of the lysosomal membrane. The invaginated membrane pinches off vesicles into the lysosomal lumen which are finally also degraded.

We discovered autophagic tubes, novel invaginations of vacuolar membranes. These vacuolar invaginations resemble those of endosomes, except for being much bigger and hence amenable to light-microscopic analysis. The invaginations pinch off vesicles into the vacuolar lumen, leading to microautophagocytosis. We have reconstituted this process in vitro with purfied vacuoles and characterize it. Scission of the vesicles, although topologically a homotypic fusion event between two vacuolar membranes, is completely independent of classical vacuolar fusion machinery. It must be driven by a novel mechanism. Freeze-fracture analyses support this. They show a striking depletion of integral membrane proteins from the nascent microautophagic vesicle. Based on this drastic lateral heterogeneity we suspect that vacuolar membrane invagination might be lipid driven and coinciding with a phase separation in the membrane. We test this hypothesis by exploring the lipid requirements of the microautophagic reaction. We could identify an enrichment of phosphatidylinositol-3-phosphate on the invaginations and a clear requirement of this lipid for vesicle formation. Furthermore, we have identified a novel role of the VTC complex in vacuolar lipid turnover that is crucial for microautophagocytosis.

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.

Fabienne Tacchini-Cottier,
Privatdocent and Senior Lecturer and Researcher

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. 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. In 2004 she was nominated Privat-Docent and Senior Lecturer and Researcher at the Faculty of Biology and Medicine.

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 Vβ4 Vα8 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.

CURRENT PROJECTS

Group of Pr. P. Launois

1. The murine model of infection with *L. major*

Role of Toll Like Receptors (TLRs) in the development of a specific adaptative response

The innate immunity, based on the non-clonal selection of specific T and B-lymphocytes, is important since it provides the early phases of host defences. The first step in innate immunity is the recognition of receptors. TLRs are a family of receptors (eleven described so far) that recognised a broad spectrum of ligands including lipids (LPS and lipoproteins), proteins (flagellin), nucleic acids and profilin. Signalling through these receptors can trigger different responses:

- They mediate internalisation of microbes by phagocytic cells
- They active anti-microbial killing mechanisms
- They induce the production of inflammatory cytokines and chemokines that can act on the development of the adaptive immune responses.

The role of TLR in innate immune response to bacterial pathogen is well recognised but very little is known about the role of TLRs during infections with parasites.

We are using different approaches to define the role of TLRs in the development of Th cell responses during infection with *L. major*.

Using real time RT-PCR, we have analyzed mRNA expression of TLRs at different time after infection with *L. major*. In resistant C57BL/6 mice, we observed that, within one day after infection, TLR7 and 9, and to a lesser

Center members 2005

Yazmin Hauyon, Technician
Chantal Pavlin-Dussauge, Apprentice
Catherine Ronet, Postdoctoral fellow
Cindy Allenbach, Ph.D student
Mélanie Breton, Ph.D student
Cynthia Perez, Trainee
Beatis Mastelic, Master student
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Diego Goyeneche, WHO trainee
Silvia Zambrana-Santander, WHO trainee
Françoise Flejszman, Secretary

extend TLR1, 2, 3 and 4 were up-regulated whereas TLR5 was down-regulated. In susceptible mice no TLRs modulation occurred. Interestingly, TLR7 and 9, were up-regulated in C57BL/6 mice 8 hours after infection and then progressively decreased later on. Since TLR7 and 9 are both intracellular receptors, this result suggest that parasites are already internalized, degraded and recognized by these receptors as soon as 8 hours after infection. Thus, recognition of parasites by extracellular occurs earlier to induce such up-regulation of TLR7 and 9 expression perhaps via extracellular TLRs.

Our results are in accordance with the fact that TLRs signalling pathway play a major role in instruction of Th1 response. To confirm the role the upregulation of TLR7 and TLR-9 up regulation in the development of Th1 cell response in resistant C57BL/6 mice, C57BL/6 mice deficient for TLR-7 and TLR-9 are currently infected and time course of infection and cell development induced by the infection analysed.

Early production of cytokine is important for Th differentiation. Indeed, neutralization of IFN-γ or IL-12 early during infection renders resistant mice susceptible to infection with *L. major* and neutralsiation of IL-4 in susceptible mice renders them resistant to infection. Concerning TLRs expression, it is known that cytokines are able to modulate TLRs expression. Thus, the role of cytokine produced early during infection with *L. major* (IL-4 in susceptible mice and IFN-γ in resistant mice) on the TLR mRNA expression was demonstrated by neutralizing cytokines with specific mAbs. One day after infection, TLR9 and TLR7 mRNA expression was shlightly decreased in C57BL/6 mice treated with anti-IFN-γ at the onset of infection as compared to non treated C57BL/6 mice.

2. The human infection

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 (*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. To this end, we have developed close collaborations with different institutions from *Leishmania* endemic countries such as Colombia, French Guiana, Sudan, and Tunisia.

Recent publications

Kariminia, A., Bourreau, E., Pascalis, H., Couppie, P., Sainte-Marie, D., Tacchini-Cottier, F., and Launois, P., (2005).
Transforming growth factor beta 1 production by CD4+ CD25+ regulatory T cells in peripheral blood mononuclear cells from healthy subjects stimulated with Leishmania guyanensis.
Infect Immun. 73, 5908-5914.

Tacchini-Cottier, F., Allenbach, C., Otten, L.A., and Radtke, F. (2004).
Notch1 expression on T cells is not required for CD4+ T Helper differentiation.
Eur. J. Immunol. 34, 1588.

Tacchini-Cottier, F., and Launois, P. (2004).
Importance of neutrophils in the regulation of Leishmania major specific immune response.
In Recent Research Developments in Immunology, Vol. 6. S.G. Pandalai, ed. Research Signpost, Kerala, p. 261.

Chakour, R., Guler, R., Bugnon, M., Allenbach, C., Garcia, I., Mauel, J., Louis, J., and Tacchini-Cottier, F. (2003).
Both the Fas ligand and inducible nitric oxide synthase are needed for control of parasite replication within lesions in mice infected with Leishmania major whereas the contribution of tumor necrosis factor is minimal.
Infect. Immun. 71, 5287.

Bourreau, E., Gardon, J., Pradinaud, R., Pascalis, H., Prevot-Linguet, G., Kariminia, A., and Launois, P. (2003).
Th2 responses predominate during the early phase of infection in patients with localized cutaneous leishmaniasis and precede the development of Th1 response.
Infect. Immun. 71, 2244-2246.

Aseffa, A., Gumy, A., Launois, P., MacDonald, H.R., Louis, J.A., and Tacchini-Cottier, F. (2002).
The early IL-4 response to Leishmania major and the resulting Th2 cell maturation steering progressive disease in BALB/c mice are subject to the control of regulatory CD4+CD25+ T cells.
J. Immunol. 169, 3232.

Bourreau, E., Collet, M., Prevot, G., Milon, G., Ashimoff, D., Hasagewa, H., Parra-Lopez C., and Launois, P. (2002).
IFN-gamma-producing CD45RA+CD8+ and IL-10-producing CD45RA-CD4+ T cells generated in response to LACK in naive subjects never exposed to Leishmania.
Eur. J. Immunol. 32, 510.

Group of PD Dr 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 PMNs with macrophages and 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 but they were also reported to induce PMN apoptosis by an undefined mechanism. To investigate if there existed differences in the induction of PMN apoptosis by macrophages from susceptible (BALB/c) or resistant (C57BL/6) mice, we first performed *in vitro* experiments in which PMNs isolated following recruitment i.p. either with 5% starch or with *L. major*, were co-cultured with increasing numbers of similarly derived macrophages. PMN apoptosis was assessed 24 hours after the initiation of co-cultures. An increase in the proportion of macrophages in the co-cultures induced a significant increase in apoptosis of C57BL/6 PMNs and to a lesser extent of BALB/c PMNs. We further showed that macrophage-induced PMN apoptosis was dependent on 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. In addition, *L. major* by itself did not induce or only very low level of PMN apoptosis, however, in presence of macrophages, *L. major* exacerbated macrophage-induced apoptosis in both C57BL/6 and BALB/c neutrophils. Our results emphasize the importance of mTNF and macrophage-induced PMN apoptosis in the early control of inflammation subsequent to an infection. (*Allenbach et al., 2006 Manuscript submitted*).

Differential production of cytokines by PMNs 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 inflammation. 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). PMN 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.

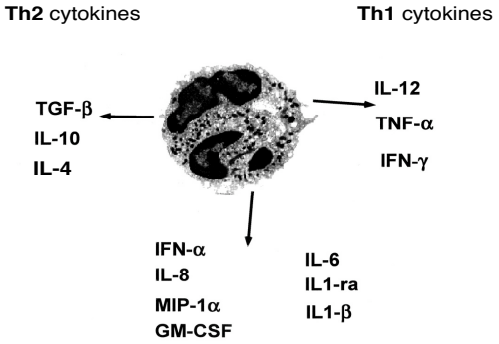


Figure 1: *Cytokines production by PMN. Cytokines involved in CD4⁺Th1 or Th2 differentiation or function have been reported to be secreted by neutrophils.*

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. A distinct pattern of IL-12 secretion was measured in *L. major*-triggered BALB/c *versus* C57BL/6PMNs. Although BALB/c PMNs secreted high levels of IL-12p40, only *L. major*-triggered C57BL/6 neutrophils secreted the biologically active IL-12p70 form. In addition, biologically active IL-12p70 was already secreted by unstimulated BM-derived C57BL/6 PMNs but not in BM-derived BALB/c PMNs, arguing for the storage and rapid release of IL-12p70 in C57BL/6 but not BALB/c PMN. No secretion of IFN-γ was observed in all the conditions tested. We are currently pursuing these studies to better understand the contribution of PMNs to the local microenvironment shaping the immune response to *L. major* parasite.

Manfredo Quadroni, Tenured Senior Lecturer and Researcher

Interests: proteomics technologies and applications to characterise protein complexes, subcellular compartments and post-translational modifications.
PhD in Biochemistry at the ETH Zürich in 1998 on calcium-mediated signalling, protein phosphorylation and applications of mass spectrometry to protein chemistry.
Post-doc at the University of British Columbia, Canada in the group of Prof. J. Schrader, with focus on the analysis of cell signalling complexes in immunology by proteomics techniques.
Second post-doc at ETH Zürich, (1998-2000) working on the development of methods for proteome analysis.
He joined the Department of Biochemistry in February 2000.

Group members 2005

Jachen Barblan, Technician
Alexandra Potts, Technician
Willy Bienvenut, Postdoctoral fellow
Bastienne Jaccard, Ph.D student
Gnanasekaran Thoppae, bioinformatician

Technologies for functional proteomics and their applications

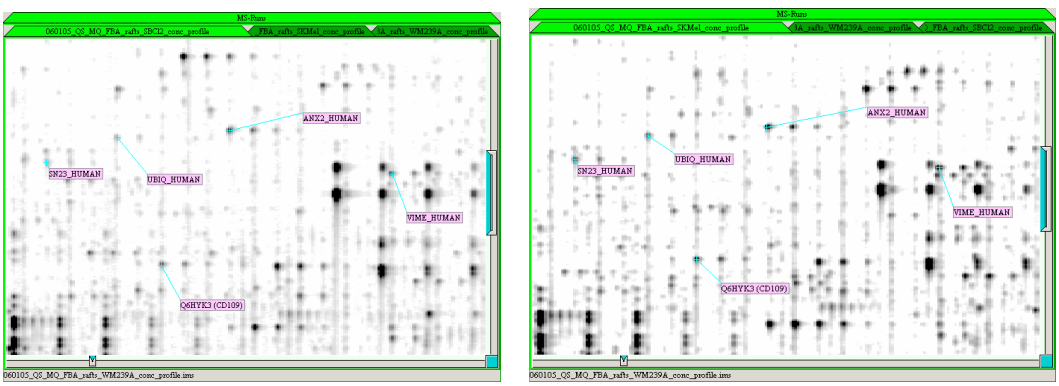
Proteomics is becoming a viable approach to study the organisation of complex cellular pathways. Together with other functional genomics techniques, proteomics is a branch of systems biology, a discipline that attempts to analyse living systems in a more global fashion than classical biology. By combining labelling and separation techniques with mass spectrometry, it is now possible to analyse complex protein mixtures to determine their composition and detect changes associated with a given biological process. This approach is most promising to analyse fractions of proteins that are connected by a functional relationship, typically by direct interaction (formation of a supramolecular complex) or co-localisation to a functionally defined cellular compartment.

Topics :

1) **technologies for the differential analysis of complex protein mixtures and their applications.** We actively pursue the development and optimisation of strategies to perform characterisation and comparison of complex protein samples both at the qualitative and quantitative level.
2) **the Protein Analysis Facility (PAF): a core facility for the Lausanne academic community.** Our group provides the local community with a service of protein identification by mass spectrometry (MALDI- or LC-ESI-MS) as well as separation and differential analysis of proteomes by two-dimensional electrophoresis (2D-PAGE). A dedicated web site is available at www.unil.ch/paf.

Current projects

Software development : MSIGHT
We have collaborated with the Swiss Institute of Bioinformatics in Geneva (Group of R. Appel) to create and test a software for the representation and comparison of liquid-chromatography-mass spectrometry data. MSIGHT, now available as a freeware (www.expasy.org/MSight/) can display and compare LC-MS and LC-MS/MS data as two-dimensional density maps. This software allows to have an immediate overview of extremely complex datasets. More importantly, it allows the analyser to exploit the correlation between mass and elution time to align and compare samples to accurately quantify peak intensities and determine significant differences.



Aligning and comparing LC-MS runs by two-dimensional display with MSIGHT

Recent publications

Grill, B., Wilson, G.M., Zhang, K.X., Wang, B., Doyonnas, R., Quadroni, M., and Schrader, J.W. (2004).
Activation/division of lymphocytes results in increased levels of Cytoplasmic activation/proliferation-associated protein (Caprin)-1: prototype of a new family of proteins.
J. Immunol. 172(4), 2389-2400.

Jousson, O., Léchenne, B., Bontems, O., Capoccia, S., Mignon, B., Barblan, J., Quadroni, M., and Monod, M. (2004).
Multiplication of an ancestral gene encoding secreted fungalysin preceded species differentiation in dermatophytes.
Microbiology 150, 301-310.

Palagi, P.M., Walther, D., Quadroni, M., Catherinet, S., Burgess, J., Zimmermann-Ivol, C.G., Sanchez, J.C., Binz, P.A., Hochstrasser, D.F., and Appel, R.D. (2005).
MSight: an image analysis software for liquid chromatography-mass spectrometry.
Proteomics 5, 2381-2384.

Arrigoni, G., Resjö, S., Levander, F., Nilsson, R., Degerman, E., and Quadroni, M. (2005).
Chemical derivatisation of phospho-serine and -threonine containing peptides to increase sensitivity for MALDI based analysis and for selectivity of MS/MS analysis.
Proteomics, in press.

Owen, H.R., Quadroni, M., Bienvenut, W., Buerki, C., and Hottiger, M.O. (2005).
Identification of novel and cell type enriched cofactors of the transcription activation domain of RelA (p65 NF-kappaB).
J. Proteome Res. 4, 1381-1390.

Metabolic labelling and specific mass spectrometry to study newly synthesized proteins
(In collaboration with the group of J.-J. Diaz and A. Greco (INSERM Lyon)
We are pursuing the development of a technique to specifically identify in complex mixtures such as whole cell extracts the proteins that were synthesized at high rates during a given time. This approach will be based on metabolic labelling of cell cultures with stable isotope derivatives of amino acids and a specific detection by mass spectrometry of fragments of these proteins that have incorporated the label. We have preliminary evidence showing the viability of the approach in a biological system (infection of cells with Herpes simplex virus).

Identification of novel and cell type enriched cofactors of the transcription activation domain of RelA (p65 NF-kappaB)
(In collaboration with the group of M. Hottiger, University of Zuerich)
To find new cell type specific cofactors of NF-kappaB dependent transcription, we isolated RelA transcription activation domain binding proteins from the nuclear extracts of three different cell types. Analysis by electrophoresis and liquid chromatography tandem mass spectrometry identified several novel putative molecular partners. Some were strongly enriched in the complex formed from the nuclear extracts of specific cell types and may help explain the differences in NF-kB dependent gene activation patterns observed among different cells.

Characterisation of molecules interacting with novel death-domain-containing proteins
(In collaboration with the group of J. Tschopp, Dept of Biochemistry, University of Lausanne)
We are studying the set of proteins specifically co-purifying with novel death domain-containing proteins such as the Caspase-2 activator PIDD and ZUDD (ZU5 and death domain-containing inhibitor of NF-kB), to gain insight into the pathways in which these molecules play a role. In addition we were able to highlight a surprising post-translational modification that both of these molecules undergo that appears to be essential for their activity.

The secretome of *Trichophyton rubrum*
(In collaboration with the group of M. Monod at the CHUV, Lausanne)
Secreted proteins constitute potential virulence factors of dermatophytes, likely involved in invasion of skin tissues by the fungus. Building on previous work, we are systematically analysing the pool of proteins secreted by *T. rubrum* and closely related species. Several types of proteases seem to be dominantly and consistently expressed and secreted by these fungi, together with hydrolytic enzymes belonging to other functional classes.

Comparative analysis of lipid rafts from melanoma cells
(In collaboration with the group of C. Ruegg, Centre Pluridisciplinaire d'Oncologie, Lausanne)
Melanoma cells derived from tissues at different stages of tumor progression display different growth patterns and metastatic phenotypes. To be able to migrate and invade other tissues, signalling and adhesion molecules such as those present in lipid rafts on the plasma membrane probably play a pivotal role. We have chosen to analyse rafts proteins from methastatic vs. non-methastatic cell lines by automated LC-MS/MS after proteolysis, with the aim to perform both qualitative and quantitative comparison of protein contents. For this we are exploiting the MSIGHT software tool that we have contributed developing. We have been able to identify several proteins that appear to be differentially expressed in the two classes of cells considered.

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. Ligands and receptors of the TNF family are his major research interest.

Group members 2005

Aubry Tardivel, Research associate
Karine Ingold, Technician
Claudia Bossen, Ph.D student

Recent publications

Avalos, A.M., Arthur, W.T., Schneider, P., Quest, A.F.G., Burridge, K. and Leyton, L. (2004).
Aggregation of integrins and RhoA activation are required for Thy-1-induced morphological changes in astrocytes.
J. Biol. Chem. 279, 39139-39145.

Legler, D.F., Doucey, M.-A., Schneider, P., Chapatte, F., Bender, F.C. and Bron, C. (2005).
Differential insertion of GPI-anchored GFPs into lipid rafts of live cells.
FASEB J. 19, 73-75.

Iordanov, M., Ryabinina, O.P., Schneider, P. and Magun, B.E. (2005).
Two mechanisms of caspase 9 processing in double-stranded RNA- and virus-triggered apoptosis.
Apoptosis 10, 153-166.

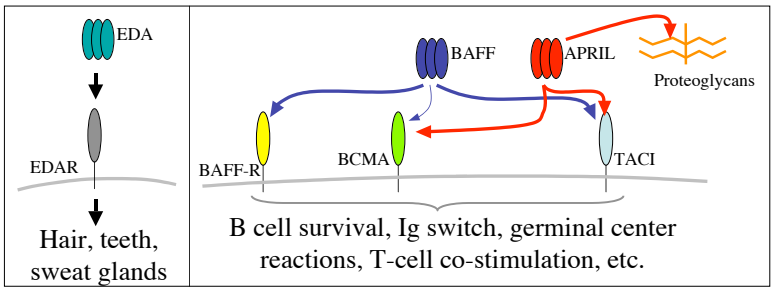
Ingold, K., Zumsteg, A., Tardivel, A., Huard, B., Steiner, Q.-G., Cachero, T., Qian, F., Gorelik, L., Kalled, S.L., Acha-Orbea, H., Rennert, P.D., Tschopp J. and Schneider, P. (2005).
Identification of proteoglycans as APRIL-specific binding partners.
J. Exp. Med. 209, 1375-1383.

Cebecauer, M., Guillaume, P., Hozak, P., Mark, S., Everett, H., Schneider, P. and Luescher, I.F. (2005).
Soluble MHC-peptide complexes induce rapid death of CD8+ CTL.
J. Immunol. 174, 6809-6819.

Functional characterization of TNF family ligands

Our group is interested in the functional characterization of ligands and receptors of the TNF family, with emphasis on three ligands, which function in the development of skin appendages (EDA) or in the immune system (BAFF and APRIL).

Mutations of EDA are causative of XLHED, a rare, incurable human genetic disorder characterized by defective formation of hair, teeth and sweat glands. We have shown previously that short-term administration of recombinant EDA in EDA-deficient mice cured the disease when the protein was administered *in utero* (non-invasively, through hijacking of the placental antibody transport system). Based on this proof of concept, our aim is to develop a treatment for humans.
BAFF is a master B cell survival factor and its close homologue, APRIL, is also important for the function of B cells. APRIL and BAFF share two receptors, TACI and BCMA, but APRIL does not bind to BAFF-R that is specific for BAFF only (Figure 1). Five years ago, we postulated the existence of an APRIL-specific "receptor", because APRIL binds to a variety of cells devoid of TACI and BCMA. Recently, we have found that basic residues of APRIL are required to bind the APRIL-specific "receptor", but not BCMA and TACI that display a distinct binding site. This novel APRIL interactor was identified as negatively charged sulfated glycosaminoglycan side chains of proteoglycans (Figure 1). As costimulation of B cell proliferation by APRIL was only effective upon its oligomerization, we therefore proposed that the binding of soluble APRIL to the extracellular matrix or to proteoglycan-positive cells might activate APRIL by inducing its oligomerization.



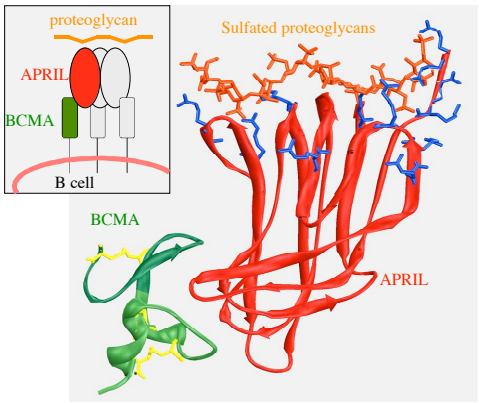


Figure 1: *EDA, BAFF and APRIL. EDA controls formation of ectodermal appendages during development. BAFF and APRIL play important roles in the biology of B cells, and to a lesser extent T cells, through engagement of various receptors. The interaction of APRIL with sulfated proteoglycans involves basic residues (in blue) and occurs at a site distinct from the binding site to BCMA and TACI. In the figure, the sulfated proteoglycan was docked manually on the crystal structure of the APRIL-BCMA complex (PDB atomic coordinate file 1XU2).*

Iordanov, M., Sundholm, A.J., Simpson, E.L., Hanifin, J.M., Ryabinina, O.P., Choi, R.J., Korcheva, V.B., Schneider, P. and Magun, B.E. (2005).
Cell death-induced activation of epidermal growth factor receptor in keratinocytes: implications for restricting epidermal damage in dermatitis.
J. Invest. Dermatol. 125, 134-142.

Salzer, U., Chapel, H.M., Webster, A.D.B., Pan-Hammarström, Q., Schmitt-Graeff, A., Schlesier, M., Peter, H.H., Rockstroh, J.K., Schneider, P., Schäffer, A.A., Hammarström, L. and Grimbacher, B. (2005).
Mutations in TNFRSF13B, which encodes TACI, are associated with common variable immunodeficiency in humans.
Nat. Genetics 25, 820-828.

Kvell, K., Tuan, N.H., Salmon, P., Glauser, F., Werner-Favre, C., Barnet, M., Schneider, P., Trono, D. and Zubler, R.H. (2005).
Transduction of CpG DNA-stimulated primary human B cells with bicistronic lentivectors.
Mol. Therapy 12, 892-899.

Reviews

Schneider, P. (2005).
APRIL and BAFF in lymphocyte activation.
Curr. Opinion Immunol. 17, 2-82-289.

Schneider, P. (2005).
TNF family ligands and receptors in cell death signaling.
AACR, Education Book, 279-284.

It has been shown recently that CD40L-independent immunoglobulin class switch, especially to the IgA class, was impaired in APRIL-deficient mice. These results have prompted a search for relevant mutations in patients with common variable immunodeficiency (CVID) and selective IgA deficiency, which revealed that several of these patients displayed mutations in TACI (collaboration with Dr B. Grimbacher). These findings demonstrate the relevance of BAFF and APRIL in the function of human B cells.

Catherine Servis, Project leader

Catherine Servis studied Biochemistry at the University of Heriot-Watt in Edinburgh and received her Ph.D. from the same University in 1986 for studies on the localization, identification and synthesis of immunodominant determinants on porcine Lactate dehydrogenase -B4 and IgG2a myeloma protein. The research of this thesis was conducted at the Max-Planck Institute for Immunogenetics in Tübingen and at the Biochemistry Department of Northwestern University in Chicago. Her postdoctoral research was carried out at the Basel Institute for Immunology (Hoffman-La-Roche), the Friedrich-Miescher Institute (Ciba-Geigy) and the Institute of Organic Chemistry at the University on Lausanne on studies related to protein structure and function. She joined the Department of Biochemistry in 1992 as Junior Faculty member. She is responsible for the Protein and Peptide Chemistry Facility at the BIL Biomedical Research Centre since 1992. She has been a member of the NCCR program since 2001 focusing on the identification and analysis of new peptide tumor antigens. She is head of the Clinical Tumor Proteome Analysis Facility since May 2005.

Facility members 2005

Nicole Lévy, Technician
Florela Penea, Technician

Protein and Peptide Chemistry Facility

What we offer

The objective of this facility is to provide information on selected aspects of peptide synthesis and protein structure and to create the resources to investigators to extend their capabilities on peptide synthesis, peptide/protein purification and mass spectroscopic analysis through the aid of protein chemists operating this facility.

SERVICES

The range of services provided by the Protein and Peptide Chemistry Facility are listed below:

Synthesis

- Peptide synthesis (linear or branched peptides)
- Peptide modification
- Peptide coupling to proteins

Purification

- Peptide purification
- Protein purification

Analysis

- Amino acid analysis
- Mass spectroscopic analysis
  1. Peptides
  2. Proteins
  3. Identification of naturally processed peptide antigens. Natural peptides extracted from antigen presenting cells by acid extraction are separated by HPLC and then identified by MS
  4. C-terminal sequencing. Peptides up to 3,000 da are digested by carbosypeptidases and the digestion products are identified by MS
  5. Identification and characterization of proteins

EQUIPMENT

Equipment currently in place include:

- Peptide synthesis is performed using two automated Applied Biosystems 431A and 433A peptide synthesizers (up to 0.2 mmol) and an ACT348 OMEGA Advanced ChemTech multiple synthesizer (0.025 and 0.1 mmol), both employing Fmoc chemistry. Standard synthesis provides 20-100 mg of peptide with are delivered within two-three weeks. Quality control includes analytical HPLC and mass spectroscopic analysis.
- High Performance Liquid Chromatography (HPLC) is performed by three Waters HPLC systems for analytical or preparative HPLC and a Hewlett Packard HPLC system for analytical applications.
- Mass spectroscopic analysis is performed by a Voyager-DE Biospectrometry Workstation by Applied Biosystems and a 4700 Proteomics Analyzer MALDI- TOF/TOF by Applied Biosystems.

Recent publications

Barcelo-Batlori, S., André, M., Servis, C., Lévy, N., Hausel, P., Knipp, M., Takikawa, O., and Felley-Bosco, E. (2002). Characterisation of cytokine-regulated proteins in a model of human inflammatory bowel disease by functional proteomics. Proteomics J. 2, 551-560.

Ayyoub, M. ,Stefanovic, S., Sahin, U., Guillaume, P., Servis, C., Rimoldi, D., Valmori, D., Romero, P., Cerottini, J-C., Rammensee, H-G., Pfreundschuh, M., Speiser, D., and Lévy, F. (2002). Proteasome-assisted identification of a SSX-2 derived epitope recognized by tumour reactive cytosolic T lymphocytes infiltrating metastatic maelanoma. J. Immunol. 168, 1717-1722.

Rubio-Godoy, V., Ayyoub, M., Dutoit, V., Servis, C., Schink, A., Rimoldi, D., Romero, P., Cerottini, J-C., Speiser, D., Simon, R., Zhao, Y., Houghten, R. A., Pinilla, C., and Valmori, D. (2002). Combinatorial peptide library based identification of peptide ligands for a tumor- reactive $CD8^+$ T cell clone of unknown specificity. Eur. J. Immunol. 32, 2292-2299.

Lévy, F., Burri, L., Morel, S., Peitrequin, A.-L., Lévy, N., Bachi, A., Hellman, U., Van den Eynde, B., and Servis, C. (2002). The N-terminal trimming of an HLA class I-restricted antigenic peptide precursor is mediated by the sequential action of two cytosolic peptidases. J. Immunol. 169, 4161-4171.

Burri, L., Servis, C., Chapatte, L., and Lévy, F. (2002). A recyclable assay to analyse the $-NH_2$ terminal trimming of antigenic peptide precursors. Protein Expres Purif. 26, 19-27.

Vuadens, F., Crettaz, D., Scelatta, C., Servis, C., Quadroni, M., Bienvenut, W-V., Schneider, P., Hohlfeld , P., Applegate, L.A., and Tissot, J-D. (2003). Plasticity of protein expression during cluture of fetal skin cells. Electrophoresis 24, 1281-1291.

Chapatte, L., Servis, C., Valmori, D., Burlet-Schiltz, O., Dayer, J., Monsarrat, B., Romero, P., and Lévy, F. (2004). Final Antigenic Melan-A Peptides Produced Directly by the Proteasomes Are Preferentially Selected for Presentation by HLA-A*0201 in Melanoma Cells. J. Immunol. 173, 6033-6040.

Research Projects

Identification of candidate 'signatures' associated with the presence of tumor angiogenesis in cancer patients

In collaboration with Prof. Curzio Rüegg (Centre Pluridisciplinaire d'Oncologie, UNIL)

The post-genome era of molecular medicine is rapidly moving beyond transcriptomics, gene lists, and functional genomics to proteomics. The function and inter-linking of proteins is directly linked to the cellular, tissue, and physiological microenvironment. The protein-protein interactions that drive complex biological processes can be characterized as a fluctuating information flow within the cell, and throughout the organism through protein pathways and the cellular protein "circuitry". The deranged molecular networks in cancer are not confined to the diseased cell, but extend out to the microenvironment of the tumor-host interface, the surrounding stromal and vascular compartments, and outward to the circulation macroenvironment. Tumor angiogenesis, is a stromal reaction essential for tumor progression. Inhibition of tumor angiogenesis suppresses tumor growth in many experimental models. The recognition that cancer is a product of the proteomic tissue microenvironment has important clinical implications from both an early detection and therapeutic targeting point of view. Many approaches to quantify tumor angiogenesis in patients in a non-invasive and reliable manner have been proposed and investigated, including the determination of tumor microvasculature density (vascular 'hot spots'), circulating angiogeneic growth factors and 'angiogenesis-associated' molecules, circulating endothelial cells and bone marrow-derived angiogenic cells, and imaging techniques. To date, however, there is still no validated marker or method available for routine use in clinical oncology.

In order to identify candidate 'signatures' associated with the presence of tumor angiogenesis in cancer patients, we analyzed serum samples of tumor patients undergoing surgery. In this pilot experiment performed in collaboration with Ciphergen we have analyzed 68 serum samples obtained from patients with non-metastatic breast or colon cancer collected before and after surgery. Analysis of these samples resulted in the identification of potential biomarkers (18 in breast and 15 in colon) distinguishing patients before treatment (with vs. without tumor, $P \leq 0.05$). This experiment demonstrated that samples obtained from patients within a NCCR study could be analyzed using a commercial clinical proteomic platform and that significant differences associated with the presence of the tumor were observed. The relevance of these differences and the identity of the peptides and proteins underlying these differences are currently being investigated. These data will be used as a 'reference' and compared with locally-generated results using samples collected within the second, ongoing colon cancer study (NCCR). Different chromatography methods for sample preparation and enrichment will be used and compared for their ability to generate the most informative spectra. In particular, we are using results obtained from the single biomarker study to guide the identification of 'angiogenic' signatures.

Spectra obtained from these experiments will be analyzed in collaboration with the bioinformatics core facility (Dr. Mauro Delorenzi) for the presence of patterns that can discriminate tumor-bearing patients from operated (tumor-free) patients and non-tumor patients.

Recent publications

Suarez, M., Haenni, M., Canarelli, S., Fisch, F., Chodanowski, P., Servis, C., Michielin, O., Freitag, R., Moreillon, P., and Mermod, N. (2005).
Structure-function characterization and optimization of a plant-derived antibacterial peptide.
Antimicrob. Agents Chemother. 49, 3847-3857.

Chapatte, L. , Ayyoub, M., Morel, S. Peitrequin, A-L., Lévy, N., Servis C., Van den Eynde, B.J., Valmori, D., and Lévy, F. (2005).
Processing of tumor-associated antigen by the proteasomes of dendritic cells controls in vivo T cell responses.
Submitted Nov 2005 J. Immunol.

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)

In collaboration with Frédéric Lévy (Ludwig Institute for Cancer Research, Lausanne Branch)

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 are being studied.

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.

Group members 2005

Montserrat Guzzardi, Technician
Sophie Turcotte, Technician
Daniel Rueda, Postdoctoral fellow
Roger Moser, Ph.D student
Myriam Tapernoux, Ph.D student
Fabien Rebeaud, Ph.D student
Jannick Petremand, Masters student

Molecular mechanisms of T-cell activation and survival

T lymphocytes play a crucial role in the defense against pathogens and tumor cells. They become activated upon triggering of the T-cell antigen receptor by MHC-bound antigen. This leads to the initiation of multiple signaling pathways that regulate gene transcription, subsequent cytokine production and cellular proliferation. Following antigen-dependent clonal expansion of the reactive T-cell pool, most of the T cells are eliminated by a programmed form of cell death (apoptosis) that is a consequence of the activation-induced up-regulation of pro-apoptotic proteins and concurrent down-regulation of anti-apoptotic proteins.
The goal of our research is to elucidate the signaling pathways that control T-cell activation and apoptosis, and to understand their molecular dysfunctions underlying the pathogenesis of diseases with altered immune functions.

T-cell receptor induced activation of the NF-κB pathway

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 have been identified as T- and B-cell specific PKC family members essential for antigen receptor-induced NF-κB activation, but the molecular mechanisms linking these kinases and the IKK complex have remained a mystery. Recently, we and others have identified the proteins Carma1, Bcl10 and Malt1 as signaling components that act downstream of the PKCs and upstream of the IKK complex, and that are essential for the activation and proliferation of lymphocytes (Fig. 1).

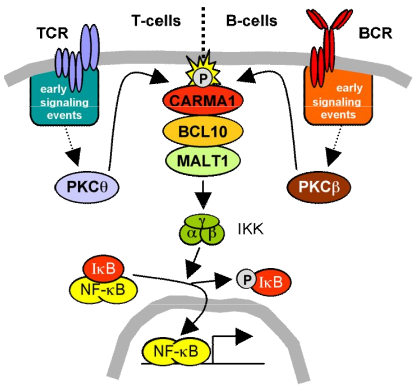


Figure 1: *T-cells are activated upon engagement of the T-cell receptor by MHC-bound antigen on the surface of the antigen-presenting cell, while B-cells are triggered by binding of multivalent antigen to their cell surface immunoglobulin receptor. This leads to the initiation of early signaling events that involve tyrosine kinases and their substrates, which in turn control the activation of Ser/Thr kinases of the protein kinase C family. The proteins Carma1, Bcl10 and Malt1 have recently been identified as signaling components that transmit the signal to the I-κB kinase (IKK) complex (Thome, 2004; Rueda and Thome, 2005). IKK activation leads to phosphorylation of the NF-κB inhibitor, I-κB, allowing nuclear translocation of NF-κB and initiation of NF-κB-dependent gene transcription.*

We had identified Carma1 as a caspase recruitment domain (CARD)-containing protein that is predominantly expressed in lymphocytes, binds to the adaptor protein Bcl10 upon T-cell receptor engagement and is essential for TCR-induced activation of NF-κB (Gaide et al., 2002; Thome, 2004). In normal T cells, T-cell receptor stimulation induces a physical association of Carma1 with Bcl10 and with the TCR complex (Gaide et al, 2002). In collaboration with the laboratory of Dan Littman, we could show that mice deficient in Carma1 show largely normal T-cell development but impaired activation and proliferation of mature T-cells, and impaired Bcl10 recruitment to the TCR complex (Egawa et al., 2003). More recently, in collaboration with the lab of Ed Palmer, we have described a proapoptotic function for Carma1 that requires its association with the engaged TCR complex (Teixeiro et al., 2004). In T-cells expressing a mutant TCR with an altered TCR beta chain, isolated from transgenic mice generated in Ed Palmer's laboratory, the recruitment of Carma1 to the TCR complex is strongly reduced (Fig. 2). This correlates with impaired/delayed NF-κB activation, but surprisingly, T cells proliferated normally and produced normal cytokine levels. In contrast, T-cell activation-induced cell death was significantly reduced, leading to exaggerated antigen-induced T-cell expansion and increased total T cell numbers. It is presently unknown whether this phenotype results from impaired NF-κB-dependent expression of pro-apoptotic genes such as FasL, or whether Carma1 has a specific pro-apoptotic function that awaits further characterization.

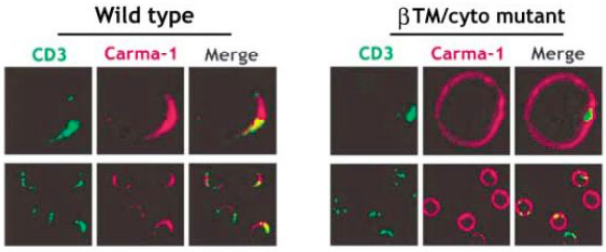


Figure 2: *In normal T cells, Carma1 is physically recruited to the TCR-CD3 complex upon triggering (here: anti-CD3 induced capping) of the receptor. Mice that transgenically express a chimeric TCR in which the transmembrane domain of the TCR beta chain has been mutated, show dramatically decreased Carma1 recruitment (Teixeiro et al., 2004).*

A role for TRAF proteins in Fas-induced cell death of lymphocytes

Upon antigen-induced clonal expansion of the T-cell pool, the majority of the activated T-cells are eliminated by a phenomenon that is called activation-induced cell death (AICD), a form of inducible T-cell apoptosis that is in part mediated by death receptors and their ligands expressed on the T-cell surface. The death receptor Fas and its ligand, FasL, play an essential role in AICD, since humans and mice deficient or mutated in Fas or FasL show impaired AICD and accumulation of autoreactive lymphocytes. The reason why activated T-cells are particularly sensitive to Fas-induced cell death are nor fully understood, but it is believed that NF-κB-dependent upregulation of FasL and concurrent downregulation of anti-apoptotic proteins are essential in the sensitization to apoptosis of activated versus resting T cells.

The molecular mechanism of Fas-induced apoptosis has been intensively studied. Upon triggering of the receptor by its trimeric ligand, Fas associates with the adaptor protein FADD and caspase-8, leading to dimerization-induced activation of caspase-8 and subsequent cleavage of downstream effector components. In order to better understand the molecular mechanisms that control Fas-induced apoptosis, we have decided to study as a model system a viral protein, MC159, that is present in a human poxvirus, the molluscum contagiosum virus. MC159 is a member of the Flice/caspase-8 inhibitory protein (FLIP) family that

Recent publications

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-κB activation.
Nature Immunol. 3, 836-843.

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-κB activation and lymphocyte proliferation.
Curr. Biol. 13, 1252-1258.

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.

Thurau, M., Everett, H., Tapernoux, M., Tschopp, J. and Thome, M.
The TRAF3 binding site of human molluscipox virus FLIP molecule MC159 is critical for its capacity to inhibit Fas-induced apoptosis.
Cell Death and Differentiation (in press).

Reviews:

Thome, M. and Tschopp, J. (2003)
TCR-induced NF-kB activation: a crucial role for Carma1, Bcl10 and MALT1.
Trends Immunol. 24, 419-424.

Thome, M. (2004)
Carma1, Bcl10 and Malt1 in lymphocyte development and activation.
Nature Rev. Immunol. 4, 348-359.

Rueda, D. and Thome, M. (2005)
Phosphorylation of Carma1: the link(er) to NF-κB activation.
Immunity 23, 1-3.

inhibits apoptosis by interfering with the function of caspase-8 (Thome and Tschopp, 2001). We had previously shown that MC159 inhibits Fas-induced apoptosis by binding to FADD, but the mechanism of its inhibitory action was not well understood. Through expression of wildtype and mutated forms of MC159 in a T-cell line, we could show that MC159 binds TRAF2 and TRAF3, members of the TNF receptor associated factor (TRAF) family, and that binding to these proteins was critical for its anti-apoptotic function (Thurau et al., in press). The intact TRAF binding site in MC159 was essential for full inhibition of caspase-8 activation and downstream cleavage events, but also for inhibition of Fas endocytosis, recently identified as a crucial event in Fas-mediated induction of apoptosis. Moreover, MC159 also inhibited Fas-induced necrosis, a caspase-independent form of cell death that may account for part of the activation-induced cell death of T cells.

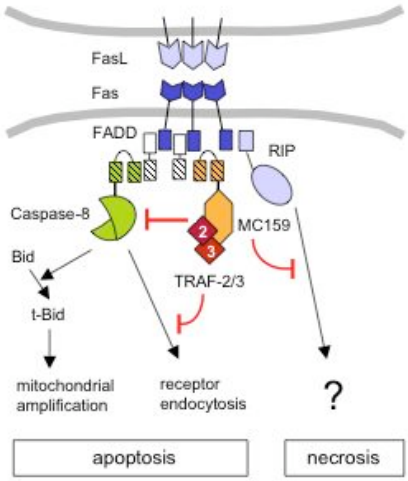


Figure 3: *Triggering of the death receptor Fas by trimeric, membrane-bound FasL induces the death of the activated T cells by apoptotic and necrotic mechanisms. The viral FLIP family member MC159 prevents Fas-induced apoptosis by recruitment of TRAF2 and –3, and provides extended inhibition of receptor endocytosis, caspase-8 activation and cleavage of downstream effectors of apoptosis. MC159 also protects against Fas-induced necrosis, a form of caspase-independent cell death that depends on the activity of the Ser/Thr kinase RIP1.*

Our findings identify MC159 as a protein that targets multiple aspects of Fas-induced cell death, and identify TRAF family members as crucial signaling components that either directly participate in or indirectly target the Fas signaling pathway in T cells.

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 full professor in 1989. Since 2003 he is Co-director of the Department of Biochemistry. His present research focuses on signaling pathways that control apoptosis and inflammation.

Group members 2005

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Antoine Tinel, Postdoctoral fellow
Laetitia Agostini, Ph.D student
Solange Cuenin, Ph.D student
Leonhard Heinz, Ph.D student
Annick Mayor, Ph.D student
Etienne Meylan, Ph.D student
Valentin Swoboda, Ph.D student

Pathogen and danger-sensing platforms that trigger apoptosis or inflammation

Apoptosis is a naturally occurring process of cell suicide that plays a crucial role in the development and maintenance of multicellular organisms by eliminating superfluous or unwanted cells. 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.
The inflammatory caspase-1 and caspase-5 are involved in the proteolytic activation of IL-1ß, which activates the rapidly acting innate immune system. They are activated by a complex, called the inflammasome, which comprises NALPs, ASC, caspase-1 and caspase-5. 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 complex formed by RIG-I/Helicard and Cardif senses the presence of viral RNA and triggers an innate anti-viral response. Our goal is to understand the signaling networks that control apoptosis and inflammatory responses to pathogens and danger signals as this will provide insights into the genesis of various human diseases.

Death receptors, a subfamily of TNF receptors which induces apoptosis

Apoptosis induced by the death receptors Fas and TNFR-1 proceeds through recruitment of FADD and caspase-8 to the receptor complex. It is unclear, however, why Fas-induced cell death occurs within minutes, whereas several hours are required for the effector function of TNFR1. We found that TNFR1-induced apoptosis (in contrast to Fas) is a two-step process, mediated by two sequential signaling complexes (Fig. 1).

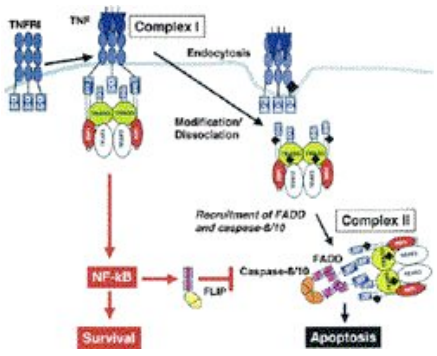

Figure 1: *After binding of TNF to TNFR1, rapid recruitment of TRADD, RIP1 and TRAF2 occurs(Complex I). Subsequently TNFR1, TRADD and RIP1 become modified and dissociate from TNFR1. The liberated death domain (DD) of TRADD (and /or RIP1) now binds to FADD, resulting in caspase8/10 recruitment (forming complex II) and resulting in apoptosis. If NF-κB activation triggered by complex I is successful,cellular FLIPL levels are sufficiently elevated to block apoptosis and cells survive.*

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. 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 a cytoplasmic complex II. In surviving

cells where NF-kB 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-kB) fails to be activated.

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

The inflammasome is a multiprotein complex (size >700 kDa) responsible for the activation of the caspases 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. 2).

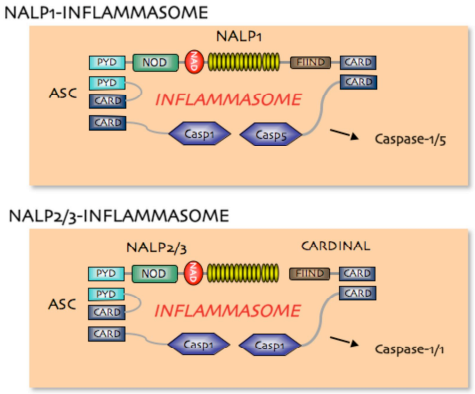

***Figure 2**: The inflammasomes*

The NALPs 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 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 pattern (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.

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. They can be successfully treated with the natural IL-1 inhibitor IL-1ra (Anakinra).

Recent publications

Micheau, O., and Tschopp, J. (2003). Induction of TNF Receptor I-Mediated Apoptosis via Two Sequential Signaling Complexes. Cell *114*, 181-190.

Meylan, E., Burns, K., Hofmann, K., Blancheteau, V., Martinon, F., Kelliher, M., and Tschopp, J. (2004). RIP1 is an essential mediator of Toll-like receptor 3-induced NF-kappa B activation. Nat. Immunol. 5, 503-507.

Agostini, L., Martinon, F., Burns, K., McDermott, E. M., Hawkins, P. N., and Tschopp, J. (2004). NALP3 forms an IL-1beta processing inflammasome with increased activity in Muckle-Wells auto-inflammatory disorder. Immunity 20, 319-325.

Tinel, A., and Tschopp, J. (2004). The PIDDosome, a Protein Complex Implicated in Activation of Caspase-2 in Response to Genotoxic Stress. Science 304, 843-846.

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.

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. 3).

Figure 3: *"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-1b 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-1b receptor (IL-1R). These findings provide insight into the molecular processes underlying the inflammatory conditions of gout and pseudogout and 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 (also known asHelicard). Both helicases detect dsRNA, and through their protein-interacting CARD domains, relay an undefined signal resulting in the activation of the transcription factors interferon regulatory factor 3 (IRF3) and NF-κB. We have recently identified Cardif, a 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 by short interfering RNA 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.

The PIDDosome: Detecting DNA damage and activating caspase-1

Activation of initiatior caspases is a key event in apoptosis execution. It is triggered upon complex-mediated clustering of the inactive zymogen as occurs in the caspase-9-activating apoptosome complex. Likewise, caspase-2, which is involved in stress-induced apoptosis, is recruited into a large protein complex, the molecular composition of which, however, was

Recent reviews

Martinon, F., and Tschopp, J. (2004).
Inflammatory caspases: linking an intracellular innate immune system to autoinflammatory diseases.
Cell 117, 561-574.

Martinon, F., Tschopp, J. (2005).
NLRs join TLRs as innate sensors of pathogens.
Trends Immunol. 26, 447-454.

Meylan, E., Tschopp, J. (2005).
The RIP kinases: crucial integrators of cellular stress.
Trends Biochem. Sci. 30, 151-159.

previously uknown. We found that activation of caspase-2 occurs in a complex that contains the death-domain containing protein PIDD, whose expression is induced by p53, and the adapter protein RAIDD. Increased amounts of PIDD expression results 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 also found that PIDD, a protein implicated in the activation of caspase-2, plays a critical role in DNA damage-induced NF-kB activation. Upon genotoxic stress, a complex between PIDD, RIP1 and NEMO is formed. Cells stably expressing PIDD show enhanced genotoxic stress-induced NF-kB activation, through augmented sumoylation and ubiquitination of NEMO. Knock-down of PIDD and RIP1 expression abrogates DNA damage-induced NEMO-modification and hence NF-kB activation. In contrast, siRNA against RAIDD and caspase-2 has no such effect, revealing the existence of two separate pathways emanating at the level of PIDD, one leading to NF-kB induction and cell survival, another to caspase-2 activation and apoptosis. We propose that PIDD acts as a molecular switch, controlling the balance between life and death upon DNA damage (Fig. 4).

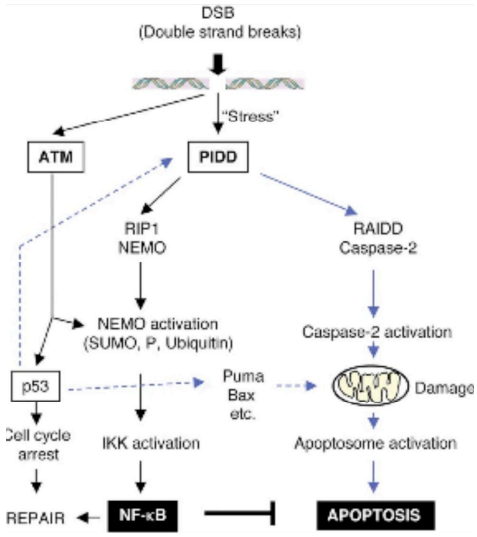

Figure 4: *PIDD acts as a molecular switch, controlling the balance between Life and Death upon DNA.*

ARTICLES

1. Agostini, L., Martinon, F., Burns, K., McDermott, M. F., Hawkins, P. N., and Tschopp, J. (2004). NALP3 forms an IL-1beta-processing inflammasome with increased activity in Muckle-Wells autoinflammatory disorder. *Immunity* **20**, 319-325.
2. Ahua, K. M., Ioset, J. R., Ransijn, A., Mauel, J., Mavi, S., and Hostettmann, K. (2004). Antileishmanial and antifungal acridone derivatives from the roots of Thamnosma rhodesica. *Phytochemistry* **65**, 963-968.
3. Anderle, P., Rumbo, M., Sierro, F., Mansourian, R., Michetti, P., Roberts, M. A., and Kraehenbuhl, J. P. (2005). Novel markers of the human follicle-associated epithelium identified by genomic profiling and microdissection. *Gastroenterology* **129**, 321-327
4. Arinola, O. G., Louis, J., Tacchini-Cottier, F., Aseffa, A., and Salimonu, L. S. (2004). Interleukin-4 (IL-4) and interferon-gamma (IFN-gamma) in pregnant C57BL/6 mice infected with L. major at different gestational periods. *West Afr J Med* **23**, 202-207.
5. Arinola, O. G., Louis, J. S., Tacchini-Cottier, F., Aseffa, A., and Salimonu, L. S. (2005). Pregnancy impairs resistance of C57BL/6 mice to Leishmania major infection. *Afr J Med Med Sci* **34**, 65-70.
6. Audran, R., Cachat, M., Lurati, F., Soe, S., Leroy, O., Corradin, G., Druilhe, P., and Spertini, F. (2005). Phase I malaria vaccine trial with a long synthetic peptide derived from the merozoite surface protein 3 antigen. *Infect Immun* **73**, 8017-8026.
7. Avalos, A. M., Arthur, W. T., Schneider, P., Quest, A. F., Burridge, K., and Leyton, L. (2004). Aggregation of integrins and RhoA activation are required for Thy-1-induced morphological changes in astrocytes. *J Biol Chem* **279**, 39139-39145.
8. Ballou, W. R., Arevalo-Herrera, M., Carucci, D., Richie, T. L., Corradin, G., Diggs, C., Druilhe, P., Giersing, B. K., Saul, A., Heppner, D. G., Kester, K. E., Lanar, D. E., Lyon, J., Hill, A. V., Pan, W., and Cohen, J. D. (2004). Update on the clinical development of candidate malaria vaccines. *Am J Trop Med Hyg* **71**, 239-247.
9. Barbey, C., Donatelli-Dufour, N., Batard, P., Corradin, G., and Spertini, F. (2004). Intranasal treatment with ovalbumin but not the major T cell epitope ovalbumin 323-339 generates interleukin-10 secreting T cells and results in the induction of allergen systemic tolerance. *Clin Exp Allergy* **34**, 654-662.
10. Batten, M., Fletcher, C., Ng, L. G., Groom, J., Wheway, J., Laabi, Y., Xin, X., Schneider, P., Tschopp, J., Mackay, C. R., and Mackay, F. (2004). TNF deficiency fails to protect BAFF transgenic mice against autoimmunity and reveals a predisposition to B cell lymphoma. *J Immunol* **172**, 812-822.
11. Bistrup, A., Tsay, D., Shenoy, P., Singer, M. S., Bangia, N., Luther, S. A., Cyster, J. G., Ruddle, N. H., and Rosen, S. D. (2004). Detection of a sulfotransferase (HEC-GlcNAc6ST) in high endothelial venules of lymph nodes and in high endothelial venule-like vessels within ectopic lymphoid aggregates: relationship to the MECA-79 epitope. *Am J Pathol* **164**, 1635-1644.
12. Buchegger, F., Adamer, F., Schaffland, A. O., Kosinski, M., Grannavel, C., Dupertuis, Y. M., de Tribolet, N., Mach, J. P., and Delaloye, A. B. (2004). Highly efficient DNA incorporation of intratumourally injected [125I]iododeoxyuridine under thymidine synthesis blocking in human glioblastoma xenografts. *Int J Cancer* **110**, 145-149.
13. Cebecauer, M., Guillaume, P., Hozak, P., Mark, S., Everett, H., Schneider, P., and Luescher, I. F. (2005). Soluble MHC-peptide complexes induce rapid death of CD8+ CTL. *J Immunol* **174**, 6809-6819.

14. Chapatte, L., Servis, C., Valmori, D., Burlet-Schiltz, O., Dayer, J., Monsarrat, B., Romero, P., and Levy, F. (2004). Final antigenic Melan-A peptides produced directly by the proteasomes are preferentially selected for presentation by HLA-A*0201 in melanoma cells. *J Immunol* **173**, 6033-6040.
15. Constantinou, A., and West, S. C. (2004). Holliday junction branch migration and resolution assays. *Methods Mol Biol* **262**, 239-253.
16. Crettaz, D., Sensebe, L., Vu, D. H., Schneider, P., Depasse, F., Bienvenut, W. V., Quadroni, M., and Tissot, J. D. (2004). Proteomics of methylene blue photo-treated plasma before and after removal of the dye by an absorbent filter. *Proteomics* **4**, 881-891.
17. 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.
18. Didierlaurent, A., Ferrero, I., Otten, L. A., Dubois, B., Reinhardt, M., Carlsen, H., Blomhoff, R., Akira, S., Kraehenbuhl, J. P., and Sirard, J. C. (2004). Flagellin promotes myeloid differentiation factor 88-dependent development of Th2-type response. *J Immunol* **172**, 6922-6930.
19. Didierlaurent, A., Ramirez, J. C., Gherardi, M., Zimmerli, S. C., Graf, M., Acha-Orbea, A., Pantaleo, G., Wagner, R., Esteban, M., Kraehenbuhl, J. P., and Sirard, J. C. (2004). Attenuated poxviruses expressing a synthetic HIV protein stimulate HLA-A2-restricted cytotoxic T-cell responses. *Vaccine* **22**, 3395-3403.
20. Dohrman, A., Kataoka, T., Cuenin, S., Russell, J. Q., Tschopp, J., and Budd, R. C. (2005). Cellular FLIP (long form) regulates CD8+ T cell activation through caspase-8-dependent NF-kappaB activation. *J Immunol* **174**, 5270-5278.
21. Dohrman, A., Russell, J. Q., Cuenin, S., Fortner, K., Tschopp, J., and Budd, R. C. (2005). Cellular FLIP long form augments caspase activity and death of T cells through heterodimerization with and activation of caspase-8. *J Immunol* **175**, 311-318.
22. Doucey, M. A., Scarpellino, L., Zimmer, J., Guillaume, P., Luescher, I. F., Bron, C., and Held, W. (2004). Cis association of Ly49A with MHC class I restricts natural killer cell inhibition. *Nat Immunol* **5**, 328-336.
23. Druilhe, P., Spertini, F., Soesoe, D., Corradin, G., Mejia, P., Singh, S., Audran, R., Bouzidi, A., Oeuvray, C., and Roussilhon, C. (2005). A malaria vaccine that elicits in humans antibodies able to kill Plasmodium falciparum. *PLoS Med* **2**, e344.
24. Germain, C., Larbouret, C., Cesson, V., Donda, A., Held, W., Mach, J. P., Pelegrin, A., and Robert, B. (2005). MHC class I-related chain a conjugated to antitumor antibodies can sensitize tumor cells to specific lysis by natural killer cells. *Clin Cancer Res* **11**, 7516-7522.
25. Gorczynski, R. M., Alexander, C., Bessler, W., Fournier, K., Hoffmann, P., Mach, J. P., Rietschel, E. T., Song, L., Waelli, T., Westphal, O., Zahringer, U., and Khatri, I. (2005). Analysis of interaction of cloned human and/or sheep fetal hemoglobin gamma-chain and LPS in augmenting induction of inflammatory cytokine production in vivo and in vitro. *Immunol Lett* **100**, 120-129.
26. Grill, B., Wilson, G. M., Zhang, K. X., Wang, B., Doyonnas, R., Quadroni, M., and Schrader, J. W. (2004). Activation/division of lymphocytes results in increased levels of cytoplasmic activation/proliferation-associated protein-1: prototype of a new family of proteins. *J Immunol* **172**, 2389-2400.
27. Gross, A., Bertholet, S., Mauel, J., and Dornand, J. (2004). Impairment of Brucella growth in human macrophagic cells that produce nitric oxide. *Microb Pathog* **36**, 75-82.

28. Grossi, M., Hiou-Feige, A., Di Vignano, A. T., Calautti, E., Ostano, P., Lee, S., Chiorino, G., and Dotto, G. P. (2005). Negative control of keratinocyte differentiation by Rho/CRIK signaling coupled with up-regulation of KyoT1/2 (FHL1) expression. *Proc Natl Acad Sci U S A* **102**, 11313-11318.
29. Guler, R., Olleros, M. L., Vesin, D., Parapanov, R., Vesin, C., Kantengwa, S., Rubbia-Brandt, L., Mensi, N., Angelillo-Scherrer, A., Martinez-Soria, E., Tacchini-Cottier, F., and Garcia, I. (2004). Inhibition of inducible nitric oxide synthase protects against liver injury induced by mycobacterial infection and endotoxins. *J Hepatol* **41**, 773-781.
30. Herrera, S., Bonelo, A., Perlaza, B. L., Fernandez, O. L., Victoria, L., Lenis, A. M., Soto, L., Hurtado, H., Acuna, L. M., Velez, J. D., Palacios, R., Chen-Mok, M., Corradin, G., and Arevalo-Herrera, M. (2005). Safety and elicitation of humoral and cellular responses in colombian malaria-naive volunteers by a Plasmodium vivax circumsporozoite protein-derived synthetic vaccine. *Am J Trop Med Hyg* **73**, 3-9.
31. Herrera, S., Bonelo, A., Perlaza, B. L., Valencia, A. Z., Cifuentes, C., Hurtado, S., Quintero, G., Lopez, J. A., Corradin, G., and Arevalo-Herrera, M. (2004). Use of long synthetic peptides to study the antigenicity and immunogenicity of the Plasmodium vivax circumsporozoite protein. *Int J Parasitol* **34**, 1535-1546.
32. Huard, B., Arlettaz, L., Ambrose, C., Kindler, V., Mauri, D., Roosnek, E., Tschopp, J., Schneider, P., and French, L. E. (2004). BAFF production by antigen-presenting cells provides T cell co-stimulation. *Int Immunol* **16**, 467-475.
33. Ingold, K., Zumsteg, A., Tardivel, A., Huard, B., Steiner, Q. G., Cachero, T. G., Qiang, F., Gorelik, L., Kalled, S. L., Acha-Orbea, H., Rennert, P. D., Tschopp, J., and Schneider, P. (2005). Identification of proteoglycans as the APRIL-specific binding partners. *J Exp Med* **201**, 1375-1383.
34. Iordanov, M. S., Ryabinina, O. P., Schneider, P., and Magun, B. E. (2005). Two mechanisms of caspase 9 processing in double-stranded RNA- and virus-triggered apoptosis. *Apoptosis* **10**, 153-166.
35. Iordanov, M. S., Sundholm, A. J., Simpson, E. L., Hanifin, J. M., Ryabinina, O. P., Choi, R. J., Korcheva, V. B., Schneider, P., and Magun, B. E. (2005). Cell death-induced activation of epidermal growth factor receptor in keratinocytes: implications for restricting epidermal damage in dermatitis. *J Invest Dermatol* **125**, 134-142.
36. Itoh, F., Itoh, S., Goumans, M. J., Valdimarsdottir, G., Iso, T., Dotto, G. P., Hamamori, Y., Kedes, L., Kato, M., and Dijke Pt, P. (2004). Synergy and antagonism between Notch and BMP receptor signaling pathways in endothelial cells. *Embo J* **23**, 541-551.
37. Janssens, S., Tinel, A., Lippens, S., and Tschopp, J. (2005). PIDD mediates NF-kappaB activation in response to DNA damage. *Cell* **123**, 1079-1092.
38. Jousson, O., Lechenne, B., Bontems, O., Capoccia, S., Mignon, B., Barblan, J., Quadroni, M., and Monod, M. (2004). Multiplication of an ancestral gene encoding secreted fungalysin preceded species differentiation in the dermatophytes Trichophyton and Microsporum. *Microbiology* **150**, 301-310.
39. Kajava, A. V., Potekhin, S. A., Corradin, G., and Leapman, R. D. (2004). Organization of designed nanofibrils assembled from alpha-helical peptides as determined by electron microscopy. *J Pept Sci* **10**, 291-297.
40. Kariminia, A., Bourreau, E., Pascalis, H., Couppie, P., Sainte-Marie, D., Tacchini-Cottier, F., and Launois, P. (2005). Transforming growth factor beta1 production by CD4(+) CD25(+) regulatory T cells in peripheral blood mononuclear cells from healthy subjects stimulated with Leishmania guyanensis. *Infect Immun* **73**, 5908-5914.

41. Kataoka, T., and Tschopp, J. (2004). N-terminal fragment of c-FLIP(L) processed by caspase 8 specifically interacts with TRAF2 and induces activation of the NF-kappaB signaling pathway. *Mol Cell Biol* **24**, 2627-2636.
42. Kunz, J. B., Schwarz, H., and Mayer, A. (2004). Determination of four sequential stages during microautophagy in vitro. *J Biol Chem* **279**, 9987-9996.
43. Kvell, K., Nguyen, T. H., Salmon, P., Glauser, F., Werner-Favre, C., Barnet, M., Schneider, P., Trono, D., and Zubler, R. H. (2005). Transduction of CpG DNA-stimulated primary human B cells with bicistronic lentivectors. *Mol Ther* **12**, 892-899.
44. 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. *Nat Med* **11**, 138-145.
45. Legler, D. F., Doucey, M. A., Schneider, P., Chapatte, L., Bender, F. C., and Bron, C. (2005). Differential insertion of GPI-anchored GFPs into lipid rafts of live cells. *Faseb J* **19**, 73-75.
46. Legler, D. F., Johnson-Leger, C., Wiedle, G., Bron, C., and Imhof, B. A. (2004). The alpha v beta 3 integrin as a tumor homing ligand for lymphocytes. *Eur J Immunol* **34**, 1608-1616.
47. LeibundGut-Landmann, S., Waldburger, J. M., Reis e Sousa, C., Acha-Orbea, H., and Reith, W. (2004). MHC class II expression is differentially regulated in plasmacytoid and conventional dendritic cells. *Nat Immunol* **5**, 899-908.
48. Luzardo-Alvarez, A., Blarer, N., Peter, K., Romero, J. F., Reymond, C., Corradin, G., and Gander, B. (2005). Biodegradable microspheres alone do not stimulate murine macrophages in vitro, but prolong antigen presentation by macrophages in vitro and stimulate a solid immune response in mice. *J Control Release* **109**, 62-76.
49. Mammucari, C., di Vignano, A. T., 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.
50. Martinon, F., Agostini, L., Meylan, E., and Tschopp, J. (2004). Identification of bacterial muramyl dipeptide as activator of the NALP3/cryopyrin inflammasome. *Curr Biol* **14**, 1929-1934.
51. Meraldi, V., Nebie, I., Tiono, A. B., Diallo, D., Sanogo, E., Theisen, M., Druilhe, P., Corradin, G., Moret, R., and Sirima, B. S. (2004). Natural antibody response to Plasmodium falciparum Exp-1, MSP-3 and GLURP long synthetic peptides and association with protection. *Parasite Immunol* **26**, 265-272.
52. Meraldi, V., Romero, J. F., Kensil, C., and Corradin, G. (2005). A strong CD8+ T cell response is elicited using the synthetic polypeptide from the C-terminus of the circumsporozoite protein of Plasmodium berghei together with the adjuvant QS-21: quantitative and phenotypic comparison with the vaccine model of irradiated sporozoites. *Vaccine* **23**, 2801-2812.
53. Meylan, E., Burns, K., Hofmann, K., Blancheteau, V., Martinon, F., Kelliher, M., and Tschopp, J. (2004). RIP1 is an essential mediator of Toll-like receptor 3-induced NF-kappa B activation. *Nat Immunol* **5**, 503-507.
54. 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.

55. Monnerat, S., Martinez-Calvillo, S., Worthey, E., Myler, P. J., Stuart, K. D., and Fasel, N. (2004). Genomic organization and gene expression in a chromosomal region of Leishmania major. *Mol Biochem Parasitol* **134**, 233-243.
56. Müller, S., Hoffmann, P., Esche, U. V., Mach, J. P., Gorczynski, R. M., Waelli, T., Alexander, C., Zahringer, U., Rietschel, E. T., Bessler, W. G., and Westphal, O. (2005). A fetal sheep liver extract containing immunostimulatory substances including LPS acts as leukocyte activator in cells of LPS responder and non responder mice. *Int Immunopharmacol* **5**, 1809-1819.
57. Mustonen, T., Ilmonen, M., Pummila, M., Kangas, A. T., Laurikkala, J., Jaatinen, R., Pispa, J., Gaide, O., Schneider, P., Thesleff, I., and Mikkola, M. L. (2004). Ectodysplasin A1 promotes placodal cell fate during early morphogenesis of ectodermal appendages. *Development* **131**, 4907-4919.
58. Nagorsen, D., Servis, C., Levy, N., Provenzano, M., Dudley, M. E., Marincola, F. M., and Levy, F. (2004). Proteasomal cleavage does not determine immunogenicity of gp100-derived peptides gp100 209-217 and gp100 209-217T210M. *Cancer Immunol Immunother* **53**, 817-824.
59. Nakhaee, A., Taheri, T., Taghikhani, M., Mohebali, M., Salmanian, A. H., Fasel, N., and Rafati, S. (2004). Humoral and cellular immune responses against Type I cysteine proteinase of Leishmania infantum are higher in asymptomatic than symptomatic dogs selected from a naturally infected population. *Vet Parasitol* **119**, 107-123.
60. Okuyama, R., LeFort, K., and Dotto, G. P. (2004). A dynamic model of keratinocyte stem cell renewal and differentiation: role of the p21WAF1/Cip1 and Notch1 signaling pathways. *J Investig Dermatol Symp Proc* **9**, 248-252.
61. Okuyama, R., Nguyen, B. C., Talora, C., Ogawa, E., Tommasi di Vignano, A., 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.
62. Owen, H. R., Quadroni, M., Bienvenut, W., Buerki, C., and Hottiger, M. O. (2005). Identification of novel and cell type enriched cofactors of the transcription activation domain of RelA (p65 NF-kappaB). *J Proteome Res* **4**, 1381-1390.
63. Palagi, P. M., Walther, D., Quadroni, M., Catherinet, S., Burgess, J., Zimmermann-Ivol, C. G., Sanchez, J. C., Binz, P. A., Hochstrasser, D. F., and Appel, R. D. (2005). MSight: an image analysis software for liquid chromatography-mass spectrometry. *Proteomics* **5**, 2381-2384.
64. Perrenoud, G., Messerli, F., Thierry, A. C., Beltraminelli, N., Cousin, P., Fasel, N., Vallet, V., Demotz, S., Duchosal, M. A., and Moulon, C. (2004). A recombinant rubella virus E1 glycoprotein as a rubella vaccine candidate. *Vaccine* **23**, 480-488.
65. Peters, C., Baars, T. L., Buhler, S., and Mayer, A. (2004). Mutual control of membrane fission and fusion proteins. *Cell* **119**, 667-678.
66. Peyre, M., Audran, R., Estevez, F., Corradin, G., Gander, B., Sesardic, D., and Johansen, P. (2004). Childhood and malaria vaccines combined in biodegradable microspheres produce immunity with synergistic interactions. *J Control Release* **99**, 345-355.
67. Philippeaux, M. M., Pache, J. C., Dahoun, S., Barnet, M., Robert, J. H., Mauel, J., and Spiliopoulos, A. (2004). Establishment of permanent cell lines purified from human mesothelioma: morphological aspects, new marker expression and karyotypic analysis. *Histochem Cell Biol* **122**, 249-260.
68. Prato, S., Maxwell, T., Pinzon-Charry, A., Schmidt, C. W., Corradin, G., and Lopez, J. A. (2005). MHC class I-restricted exogenous presentation of a synthetic 102-mer malaria vaccine polypeptide. *Eur J Immunol* **35**, 681-689.

69. Rafati, S., Salmanian, A. H., Taheri, T., Masina, S., Schaff, C., Taslimi, Y., and Fasel, N. (2004). Type I signal peptidase from Leishmania is a target of the immune response in human cutaneous and visceral leishmaniasis. *Mol Biochem Parasitol* **135**, 13-20.
70. Reese, C., Heise, F., and Mayer, A. (2005). Trans-SNARE pairing can precede a hemifusion intermediate in intracellular membrane fusion. *Nature* **436,** 410-414.
71. Reese, C., and Mayer, A. (2005). Transition from hemifusion to pore opening is rate limiting for vacuole membrane fusion. *J Cell Biol* **171**, 981-990.
72. Rumbo, M., Sierro, F., Debard, N., Kraehenbuhl, J. P., and Finke, D. (2004). Lymphotoxin beta receptor signaling induces the chemokine CCL20 in intestinal epithelium. *Gastroenterology* **127**, 213-223.
73. Salzer, U., Chapel, H. M., Webster, A. D., Pan-Hammarstrom, Q., Schmitt-Graeff, A., Schlesier, M., Peter, H. H., Rockstroh, J. K., Schneider, P., Schaffer, A. A., Hammarstrom, L., and Grimbacher, B. (2005). Mutations in TNFRSF13B encoding TACI are associated with common variable immunodeficiency in humans. *Nat Genet* **37**, 820-828.
74. Schneider, K., Kothlow, S., Schneider, P., Tardivel, A., Gobel, T., Kaspers, B., and Staeheli, P. (2004). Chicken BAFF--a highly conserved cytokine that mediates B cell survival. *Int Immunol* **16**, 139-148.
75. Sedegah, M., Charoenvit, Y., Aguiar, J., Sacci, J., Hedstrom, R., Kumar, S., Belmonte, A., Lanar, D. E., Jones, T. R., Abot, E., Druilhe, P., Corradin, G., Epstein, J. E., Richie, T. L., Carucci, D. J., and Hoffman, S. L. (2004). Effect on antibody and T-cell responses of mixing five GMP-produced DNA plasmids and administration with plasmid expressing GM-CSF. *Genes Immun* **5**, 553-561.
76. Singh, S., Soe, S., Mejia, J. P., Roussilhon, C., Theisen, M., Corradin, G., and Druilhe, P. (2004). Identification of a conserved region of Plasmodium falciparum MSP3 targeted by biologically active antibodies to improve vaccine design. *J Infect Dis* **190**, 1010-1018.
77. Singh, S., Soe, S., Roussilhon, C., Corradin, G., and Druilhe, P. (2005). Plasmodium falciparum merozoite surface protein 6 displays multiple targets for naturally occurring antibodies that mediate monocyte-dependent parasite killing. *Infect Immun* **73**, 1235-1238.
78. Suarez, M., Haenni, M., Canarelli, S., Fisch, F., Chodanowski, P., Servis, C., Michielin, O., Freitag, R., Moreillon, P., and Mermod, N. (2005). Structure-function characterization and optimization of a plant-derived antibacterial peptide. *Antimicrob Agents Chemother* **49**, 3847-3857.
79. Tacchini-Cottier, F., Allenbach, C., Otten, L. A., and Radtke, F. (2004). Notch1 expression on T cells is not required for CD4+ T helper differentiation. *Eur J Immunol* **34**, 1588-1596.
80. Tardivel, A., Tinel, A., Lens, S., Steiner, Q. G., Sauberli, E., Wilson, A., Mackay, F., Rolink, A. G., Beermann, F., Tschopp, J., and Schneider, P. (2004). The anti-apoptotic factor Bcl-2 can functionally substitute for the B cell survival but not for the marginal zone B cell differentiation activity of BAFF. *Eur J Immunol* **34**, 509-518.
81. Teixeiro, E., 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 TCRbeta chain constant domains. *Immunity* **21**, 515-526.
82. Thorel, F., Constantinou, A., Dunand-Sauthier, I., Nouspikel, T., Lalle, P., Raams, A., Jaspers, N. G., Vermeulen, W., Shivji, M. K., Wood, R. D., and Clarkson, S. G. (2004). Definition of a short region of XPG necessary for TFIIH interaction and stable recruitment to sites of UV damage. *Mol Cell Biol* **24**, 10670-10680.

83. Tinel, A., and Tschopp, J. (2004). The PIDDosome, a protein complex implicated in activation of caspase-2 in response to genotoxic stress. *Science* **304**, 843-846.
84. Uttenweiler, A., Schwarz, H., and Mayer, A. (2005). Microautophagic vacuole invagination requires calmodulin in a CA2+independent function. *J Biol Chem* **280**, 33289-33297
85. Wang, J., Devgan, V., Corrado, M., Prabhu, N. S., El-Deiry, W. S., Riccardi, C., Pandolfi, P. P., Missero, C., and Dotto, G. P. (2005). Glucocorticoid-induced tumor necrosis factor receptor is a p21Cip1/WAF1 transcriptional target conferring resistance of keratinocytes to UV light-induced apoptosis. *J Biol Chem* **280**, 37725-37731.
86. Wu, W., Rinaldi, L., Fortner, K. A., Russell, J. Q., Tschopp, J., Irvin, C., and Budd, R. C. (2004). Cellular FLIP long form-transgenic mice manifest a Th2 cytokine bias and enhanced allergic airway inflammation. *J Immunol* **172**, 4724-4732.

REVIEWS

1. Corbett, M., and Kraehenbuhl, J. P. (2004). Lung immunity: necessity is the mother of induction. *Nat Med* **10**, 904-905.
2. Corradin, G., and Giudice, G. (2005). Novel Adjuvants for Vaccines. *Current Medicinal Chemistry - Anti-Inflammatory & Anti-Allergy Agents* **4**, 185-191.
3. Corradin, G., Spertini, F., and Verdini, A. (2004). Medicinal application of long synthetic peptide technology. *Expert Opin Biol Ther* **4**, 1629-1639.
4. Debard, N., Py, P., Kraehenbuhl, J. P., and Fuchs, J. (2005). The influence of the Internet on immunology education. *Nat Rev Immunol* **5**, 736-740.
5. Dotto, G. P., and Cotsarelis, G. (2005). Developmental biology. Rac1 up for epidermal stem cells. *Science* **309**, 890-891.
6. Dotto, G. P., and Silke, J. (2004). More than cell death: caspases and caspase inhibitors on the move. *Dev Cell* **7**, 2-3.
7. Gumy, A., Louis, J. A., and Launois, P. (2004). The murine model of infection with Leishmania major and its importance for the deciphering of mechanisms underlying differences in Th cell differentiation in mice from different genetic backgrounds. *Int J Parasitol* **34**, 433-444.
8. Jousson, O., Lechenne, B., Bontems, O., Mignon, B., Reichard, U., Barblan, J., Quadroni, M., and Monod, M. (2004). Secreted subtilisin gene family in Trichophyton rubrum. *Gene* **339**, 79-88.
9. Kraehenbuhl, J. P., and Corbett, M. (2004). Immunology. Keeping the gut microflora at bay. *Science* **303**, 1624-1625.
10. Launois, P., and Tacchini-Cottier, F. (2004). Importance of neutrophils in the regulation of Leishmania major specific immune response. In *Recent Res Devel Immunology*, pp. 261-279.
11. 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.
12. LeibundGut-Landmann, S., Waldburger, J. M., Krawczyk, M., Otten, L. A., Suter, T., Fontana, A., Acha-Orbea, H., and Reith, W. (2004). Mini-review: Specificity and expression of CIITA, the master regulator of MHC class II genes. *Eur J Immunol* **34**, 1513-1525.
13. Martinon, F., and Tschopp, J. (2004). Inflammatory caspases: linking an intracellular innate immune system to autoinflammatory diseases. *Cell* **117**, 561-574.
14. Martinon, F., and Tschopp, J. (2005). NLRs join TLRs as innate sensors of pathogens. *Trends Immunol* **26**, 447-454.

15. Meylan, E., and Tschopp, J. (2005). The RIP kinases: crucial integrators of cellular stress. *Trends Biochem Sci* **30**, 151-159.
16. Muppidi, J. R., Tschopp, J., and Siegel, R. M. (2004). Life and death decisions: secondary complexes and lipid rafts in TNF receptor family signal transduction. *Immunity* **21**, 461-465.
17. Niedergang, F., Didierlaurent, A., Kraehenbuhl, J. P., and Sirard, J. C. (2004). Dendritic cells: the host Achille's heel for mucosal pathogens? *Trends Microbiol* **12**, 79-88.
18. Petrilli, V., Papin, S., and Tschopp, J. (2005). The inflammasome. *Curr Biol* **15**, R581.
19. Quadroni, M., Ducret, A., and Stocklin, R. (2004). Quantify this! Report on a round table discussion on quantitative mass spectrometry in proteomics. *Proteomics* **4**, 2211-2215.
20. Rueda, D., and Thome, M. (2005). Molecule page : Carma1. *AfCS nature molecule pages*. DOI:10.1038/mp.a003863.01
21. Rueda, D., and Thome, M. (2005). Phosphorylation of CARMA1: the link(er) to NF-kappaB activation. *Immunity* **23**, 551-553.
22. Rumbo, M., Anderle, P., Didierlaurent, A., Sierro, F., Debard, N., Sirard, J. C., Finke, D., and Kraehenbuhl, J. P. (2004). How the gut links innate and adaptive immunity. *Ann N Y Acad Sci* **1029**, 16-21.
23. Schneider, P. (2005). The role of APRIL and BAFF in lymphocyte activation. *Curr Opin Immunol* **17**, 282-289.
24. Thome, M. (2004). CARMA1, BCL-10 and MALT1 in lymphocyte development and activation. *Nat Rev Immunol* **4**, 348-359.

BOOK SECTIONS

1. Schneider, P. (2005). TNF family ligands and receptors in cell death signaling. In *AACR Education book*, pp. 279-284.
2. Schneider, P. (2004). Signaling by TNF and related ligands. In *Inborn errors of development*, C. Epstein, R. Erickson, and A. Wynshaw-Boris, eds. (Oxford, Oxford University Press), pp. 340-358.
3. Schneider, P. (2004). Tools for activation and neutralization of Fas signaling. In *Fas signaling*, H.Wajant, ed. (Georgetown, Eurekah.com).
4. Thome, M. (2004). Regulation of Fas signaling by FLIP proteins. In *Fas signaling*, H. Wajant, ed. (Landes Bioscience), pp. ch. 5.