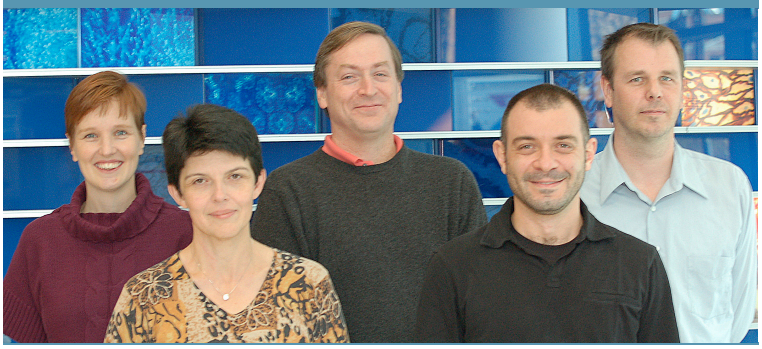
Molecular Immunology

Division Photo



E. Janssen, C. Chougnet, C. Karp, J. Aliberti, K. Hoebe

Division Data Summary

Research and Training Details

Number of Faculty	6
Number of Joint Appointment Faculty	1
Number of Research Fellows	7
Number of Research Students	4
Number of Support Personnel	11
Direct Annual Grant Support	$2,558,923
Peer Reviewed Publications	24

Clinical Activities and Training

Number of Clinical Fellows	2
Number of Other Students	2

Faculty Members

Christopher Karp, MD, Professor ; *Director, CF Research; Director, Trustee and Procter Scholar Grant Programs; Associate Director, Immunobiology Graduate Program*

Julio Aliberti, PhD, Assistant Professor

Claire A. Chougnet, PhD, Associate Professor

Kasper Hoebe, PhD, Assistant Professor

Edith M. Janssen, PhD, Assistant Professor

Joerg Koehl, MD, Adjunct Professor

Joint Appointment Faculty Members

Jonathan Katz, PhD, Associate Professor
Endocrinology

Trainees

- **Rajat Madan, BS,** GSY-7,

- **Celine Silva-Lages, PhD,** PGY-4,
- **Manoj Pandey, PhD,** PGY-4,
- **Yuan Yuan Gu, BS,** GSY-4,
- **Jessica Allen, BS,** GSY-4,
- **Naonori Uozumi, PhD,** PGY-3,
- **Fabiana Machado, PhD,** PGY-3,
- **Senad Divanovic, PhD,** PGY-2,
- **Pietro Pressice, PhD,** PGY-2,
- **Alice Nyakeriga, PhD,** PGY-2,
- **Isaac Harley, BS,** GSY-1,
- **Xun Zhang, BS,** GSY-1,

Significant Accomplishments in FY08

Recruitment of Drs. Janssen and Hoebe

A major event for the Division of Molecular Immunology in 2008 was the recruitment of two superb immunologists from San Diego—Drs. Edith Janssen and Kasper Hoebe.

The research program of Dr. Janssen focuses on mechanistic analysis and translational exploitation of novel pathway of activation of antigen-specific adaptive immune responses discovered by Dr. Janssen involving a novel class of dendritic cells (DC) and directed towards detection of antigens expressed by apoptotic cells. Sensing and clearance of apoptotic cells has generally been considered to be a non-inflammatory or even tolerizing process. While cellular antigens are a major source for antigen cross-presentation, a process in which DC capture antigens from another source and process these captured exogenous antigens into the MHC class I pathway, the prevailing view has been that the apoptotic cells generated by normal tissue turnover are captured by DC that migrate to local lymph nodes and induce T cell tolerance, anergy or deletion. Although this is an extremely useful mechanism for preventing the development of autoimmune responses, it also inhibits the induction of protective anti-tumor responses. Seminal studies by Dr. Janssen suggest that the phagocytosis of apoptotic cells by DC can also have pro-inflammatory effects that can lead to the induction of CD4+ and CD8+ T cells specific for cell-associated antigens. This balance between immune-suppressive and pro-inflammatory responses is greatly affected by the type of DC that is involved in the uptake of apoptotic cells, and the milieu that these DC subsequently create. The Janssen laboratory aims to define the molecular and cellular mechanisms in DCs that balance the pro- and anti-inflammatory immune response to self after cell death in order to facilitate translational exploitation of these mechanistic insights in the development of effective therapeutic and preventive cancer vaccines.

The research program of Dr. Hoebe is focused on developing a thorough mechanistic understanding of the connections between innate and adaptive immunity, something essential for the generation of efficient adjuvants for vaccine development. Our understanding of how adjuvants work is limited. Fundamental questions as to what pathways drive robust T and B cell responses remain largely unanswered. In addition, there is still an urgent need to find robust, safe adjuvant pathways that will lead to strong antigen-specific immune responses. The Hoebe laboratory has recently identified an “endogenous adjuvant” pathway mediated by NK cells. NK cells detect and kill pathogen-infected host cells, as well as neoplastic cells and tissue allografts. However, studies from the Hoebe laboratory have shown that they discharge another duty as well: one that establishes a strong tie between NK cells and the adaptive immune system. Of key importance in this pathway is the recognition and killing of antigen-expressing target cells by NK cells. Subsequently, NK cell-induced cell death is recognized by dendritic cells (DC), leading to antigen cross-presentation and to strong cellular and humoral immune responses. Dr. Hoebe’s laboratory is focused on obtaining a detailed understanding of the molecular requirements of this pathway by a forward genetic approach. After N-ethyl-N-nitrosourea (ENU)-mediated random germline mutagenesis, his research team is screening for mice carrying recessive phenotypic changes in NK cell function or NK cell-driven adaptive immune responses. In his hands, ENU mutagenesis has proven to be a powerful means of identifying genes with non-redundant function in innate and/or adaptive immunity—providing new insight into genes causing severe primary immunodeficiency disorders. The approach is particularly powerful in that it is unbiased: one only needs to find phenotypes, and then establish causality by positional cloning. Dr. Hoebe is convinced that the availability of the complete mouse genomic sequence, combined with the “state of the art” technology at CCHMC—including the latest high throughput sequencing technology—will tremendously accelerate the identification of single base-pair changes responsible for observed phenotypes, making the approach now more exciting than ever. Ultimately, Dr. Hoebe’s laboratory aims to exploit the knowledge obtained on NK cell-driven adaptive immune responses for the generation of new, safer vaccine approaches. To this end Dr. Hoebe has recently started collaborating with the International Aids Vaccine Initiative to construct DNA vectors to drive activation of a variety of innate immune effectors—

including NK cells.

Unraveling the mechanism of action of aspirin

Dr. Julio Aliberti's team recently published a notable paper(J Exp Med 205:1077) demonstrating the intracellular mechanisms by which a class of anti-inflammatory mediators, the lipoxins, inhibit pro-inflammatory responses. Lipoxins are endogenous lipid mediators with a wide spectrum of activities involved in the inhibition and resolution of inflammation. As such there is considerable interest in translational development of lipoxins for diverse inflammatory diseases, including cystic fibrosis. Of particular interest, the manuscript also describes a novel, critical mechanism of action for a drug that has widely used for the last 120 years— aspirin. It is known that many of the anti-inflammatory actions of aspirin are due to the endogenous generation of 15-epi-lipoxin. Native and aspirin-triggered lipoxins have identical mechanisms of action. As described by Dr. Aliberti in this manuscript, lipoxins inhibit innate immune signaling by inducing SOCS2-dependent ubiquitinylation of TRAF2 and TRAF6, adaptor molecules that couple the TNF and interleukin-1 receptor/Toll-like receptor family members to intracellular signaling events. Ubiquitinylation of TRAF2 and TRAF6 leads to the proteasomal degradation of these molecules, therefore depleting the cell of these adaptors and inhibiting pro-inflammatory cytokine production by dendritic cells. This work suggest a new molecular target for drug development for the plethora of diseases marked by dysregulated inflammatory responses.

Defining the underlying mechanisms of immunosuppression in HIV and aging

Dr. Claire Chougnet's laboratory, focused on understanding the mechanisms of T cell dysfunction associated with HIV and aging, has recently discovered that regulatory T cells (Treg cells) undergo dramatic accumulation in lymphoid organs in both settings. Because Treg cells are known to be suppressive of T cell responses, it is likely that these cells play an important role in inefficient anti-HIV immune responses seen with HIV infection, as well as in the systemic and local immune suppression seen in aging hosts. Dr. Chougnet's lab is employing experimental animal models as well as human clinical studies to define the mechanisms regulating the accumulation of Treg cells in HIV infection and aging. Recent data suggest that changes in Treg cell homeostasis— particularly increased peripheral survival—may well be essential in this process.

Significant Publications in FY08

Machado FS, Esper L, et al. (2008). "Native and aspirin-triggered lipoxins control innate immunity by inducing proteasomal degradation of TRAF6." J Exp Med 205(5): 1077-86.

This seminal paper reports novel molecular insights into the mechanism of action of lipoxins, endogenous lipid mediators important in down-modulating and resolving inflammation. In addition to suggesting a new molecular target for drug development for diseases marked by dysregulated inflammatory responses, this work eludated a hitherto unappreciated mechanism of action of aspirin.

Boasso A, Vaccari M, et al. (2007). "Regulatory T-cell markers, indoleamine 2,3-dioxygenase, and virus levels in spleen and gut during progressive simian immunodeficiency virus infection." J Virol 81(21): 11593-603.

This important paper provides *in vivo* evidence of the likely importance of increased regulatory T cell numbers and increased expression of the immunosuppressive enzyme, indoleamine 2,3-dioxygenase, whose expression in antigen presenting cells in induced by regulatory T cells, in the immunosuppression observed in non-human primate models of HIV infection.

Saxena V, Ondr JK, et al. (2007). "The countervailing actions of myeloid and plasmacytoid dendritic cells control autoimmune diabetes in the non-obese diabetic mouse." J Immunol 179(8): 5041-53.

This important paper provides new insight into the role of antigen presenting cell subsets in the pathogenesis of type I diabetes. Notably, in this mouse model, conventional myeloid dendritic cells are pathogenic—providing critical antigenic stimulation to naive CD4(+) T cells, and plasmacytoid dendritic cells are protective— providing regulatory control of CD4(+) T cell function in the pancreas.

Hoebe K, Beutler B. (2008). "Forward genetic analysis of TLR-signaling pathways: an evaluation." Adv Drug Deliv Rev 60(7):824-9.

Publication of this review provides an opportunity to announce the recruitment of the first author of this publication to CCHMC, and the research program that he brings with him. A pioneer in the use of forward genetic approaches to understanding fundamental issues in innate immunity, Dr. Hoebe's review provides an overview of the past success

of forward genetics (employing mutagenesis in mice) to decipher innate immune signaling pathways, and discusses its bright future prospects.

Division Publications

1. Bafica A, Feng CG, Santiago HC, Aliberti J, Cheever A, Thomas KE, Taylor GA, Vogel SN, Sher A. **The IFN-inducible GTPase LRG47 (Irgm1) negatively regulates TLR4-triggered proinflammatory cytokine production and prevents endotoxemia**. *J Immunol.* 2007; 179: 5514-22.
2. Kuribayashi JS, Bombardieri CR, Baracho GV, Aliberti J, Machado FS, Kalil J, Guilherme L, Kokron CM, Rizzo LV, de Camargo MM. **Slower rescue of ER homeostasis by the unfolded protein response pathway associated with common variable immunodeficiency**. *Mol Immunol.* 2008; 45: 2990-7.
3. Oliveira CJ, Cavassani KA, More DD, Garlet GP, Aliberti JC, Silva JS, Ferreira BR. **Tick saliva inhibits the chemotactic function of MIP-1alpha and selectively impairs chemotaxis of immature dendritic cells by down-regulating cell-surface CCR5**. *Int J Parasitol.* 2008; 38: 705-16.
4. Boasso A, Vaccari M, Hryniewicz A, Fuchs D, Nacsa J, Cecchinato V, Andersson J, Franchini G, Shearer GM, Chougnet C. **Regulatory T-cell markers, indoleamine 2,3-dioxygenase, and virus levels in spleen and gut during progressive simian immunodeficiency virus infection**. *J Virol.* 2007; 81: 11593-603.
5. Chougnet CA, Shearer GM. **Regulatory T cells (Treg) and HIV/AIDS: summary of the September 7-8, 2006 workshop**. *AIDS Res Hum Retroviruses.* 2007; 23: 945-52.
6. Li S, Gowans EJ, Chougnet C, Plebanski M, Dittmer U. **Natural regulatory T cells and persistent viral infection**. *J Virol.* 2008; 82: 21-30.
7. Shivakumar P, Sabla G, Mohanty S, McNeal M, Ward R, Stringer K, Caldwell C, Chougnet C, Bezerra JA. **Effector role of neonatal hepatic CD8+ lymphocytes in epithelial injury and autoimmunity in experimental biliary atresia**. *Gastroenterology.* 2007; 133: 268-77.
8. Velilla PA, Montoya CJ, Hoyos A, Moreno ME, Chougnet C, Rugeles MT. **Effect of intrauterine HIV-1 exposure on the frequency and function of uninfected newborns' dendritic cells**. *Clin Immunol.* 2008; 126: 243-50.
9. Velilla PA, Shata MT, Lages CS, Ying J, Fichtenbaum CJ, Chougnet C. **Effect of low-dose IL-2 immunotherapy on frequency and phenotype of regulatory T cells and NK cells in HIV/HCV-coinfected patients**. *AIDS Res Hum Retroviruses.* 2008; 24: 52-61.
10. Georgel P, Du X, Hoebe K, Beutler B. **ENU mutagenesis in mice**. *Methods Mol Biol.* 2008; 415: 1-16.
11. Hoebe K, Beutler B. **Forward genetic analysis of TLR-signaling pathways: an evaluation**. *Adv Drug Deliv Rev.* 2008; 60: 824-9.
12. Huang YH, Hoebe K, Sauer K. **New therapeutic targets in immune disorders: ItpkB, Orai1 and UNC93B**. *Expert Opin Ther Targets.* 2008; 12: 391-413.
13. Rutschmann S, Hoebe K. **Dissecting innate immunity by germline mutagenesis**. *Immunology.* 2008; 123: 459-68.
14. Benedict CA, Loewendorf A, Garcia Z, Blazar BR, Janssen EM. **Dendritic cell programming by cytomegalovirus stunts naive T cell responses via the PD-L1/PD-1 pathway**. *J Immunol.* 2008; 180: 4836-47.
15. Rhoads KR, Janssen EM, Luthy RG, Criddle CS. **Aerobic biotransformation and fate of N-ethyl perfluorooctane sulfonamidoethanol (N-EtFOSE) in activated sludge**. *Environ Sci Technol.* 2008; 42: 2873-8.
16. Cavuoto K, Zutshi D, Karp CL, Miller D, Feuer W. **Update on bacterial conjunctivitis in South Florida**. *Ophthalmology.* 2008; 115: 51-6.
17. Divanovic S, Trompette A, Petiniot LK, Allen JL, Flick LM, Belkaid Y, Madan R, Haky JJ, Karp CL. **Regulation of TLR4 signaling and the host interface with pathogens and danger: the role of RP105**. *J Leukoc Biol.* 2007; 82: 265-71.
18. Dragin N, Shi Z, Madan R, Karp CL, Sartor MA, Chen C, Gonzalez FJ, Nebert DW. **Phenotype of the Cyp1a1/1a2/1b1-/- triple-knockout mouse**. *Mol Pharmacol.* 2008; 73: 1844-56.
19. Karp CL, Auwaerter PG. **Coinfection with HIV and tropical infectious diseases. II. Helminthic, fungal, bacterial, and viral pathogens**. *Clin Infect Dis.* 2007; 45: 1214-20.
20. Karp CL, Auwaerter PG. **Coinfection with HIV and tropical infectious diseases. I. Protozoal pathogens**. *Clin Infect Dis.* 2007; 45: 1208-13.
21. Machado FS, Esper L, Dias A, Madan R, Gu Y, Hildeman D, Serhan CN, Karp CL, Aliberti J. **Native and aspirin-triggered lipoxins control innate immunity by inducing proteasomal degradation of TRAF6**. *J Exp Med.* 2008; 205: 1077-86.
22. Reckling S, Divanovic S, Karp CL, Wojciechowski S, Belkaid Y, Hildeman D. **Proapoptotic Bcl-2 family member Bim promotes persistent infection and limits protective immunity**. *Infect Immun.* 2008; 76: 1179-85.

23. Tsiklis NS, Kymionis GD, Karp CL, Naoumidi T, Pallikaris AI. Nine-year follow-up of a posterior chamber phakic IOL in one eye and LASIK in the fellow eye of the same patient. *J Refract Surg.* 2007; 23: 935-7.
24. Koehl J, Djulic A, Kirner V, Nguyen TT, Heiser I. Ethylene is required for elicitin-induced oxidative burst but not for cell death induction in tobacco cell suspension cultures. *J Plant Physiol.* 2007; 164: 1555-63.

Grants, Contracts, and Industry Agreements

Grant and Contract Awards — Annual Direct / Project Period Direct

Aliberti, J

Control of Immune Response by Lipoxins During Tuberculosis
National Institutes of Health
R01 AI 075038 — 02/01/08 - 01/31/13 — $200,000 / $1,000,000

Chougnet, C

CD40 Ligand Dysregulation And HIV Pathogenesis
National Institutes of Health
R01 AI 056927 — 01/01/05 - 12/31/09 — $232,541 / $1,196,252

Role of Regulatory T Cells in HIV Infection
National Institutes of Health
R01 AI 068524 — 08/01/06 - 07/31/11 — $254,362 / $1,250,504

Hoebe, K

A Novel NK Cell Mediated Adjuvant Approach to Generate CD8+ T Cell Responses
International AIDS Vaccine Initiative
05/01/08 - 12/31/08 — $194,581 / $402,439

Karp, C

Role Of Regulatory T Cells In Leishmania Major Infections
National Institutes of Health
R01 AI 057992 — 03/01/04 - 02/28/09 — $186,033 / $970,969

Role of Aeroallergen Mimics of TLR Complex Proteins in Asthma Pathogenesis
Sandler Program for Asthma Research
06-0284 — 07/01/06 - 06/30/09 — $250,000 / $750,000

Lipid Mediators And Dysregulated Inflammation In CF
National Institutes of Health
R01 HL 079312 — 04/01/05 - 03/31/10 — $311,268 / $1,569,259

Regulation of TLR Signaling and Innate Immunity by RP105
National Institutes of Health
R01 AI 075159 — 07/01/07 - 06/30/12 — $250,000 / $1,231,000

Hepatitis C Pathogenesis and the Human Genome
National Institutes of Health (Johns Hopkins University)
R01 DA 103324 — 09/01/04 - 08/31/09 — $10,056 / $48,855

Cystic Fibrosis Foundation Research Development Program
Cystic Fibrosis Foundation
09/01/07 - 08/31/09 — $180,000 / $180,000

Karp, C	Core #4	90,000
Karp, C	Project #2	40,000
Whitsett, J	Transgenic Core	50,000

Digestive Health Center - Bench to Bedside Research in Pediatric Digestive Disease - P&F #6
National Institutes of Health
P30 DK 078391 — 08/01/07 - 05/31/12 — $25,000 / $25,000

Cincinnati Rheumatic Disease Core Center - P&F#1
National Institutes of Health
P30 AR 047363 — 09/01/06 - 06/30/11 — $50,000 / $150,000

Multicenter Investigation of Genetic Modifiers in CF Lung and Liver
Cystic Fibrosis Foundation (University of North Carolina)
12/15/05 - 12/31/08 $2,625 / $7,875

Koehl, J

Complement In Allergic Asthma: The Role Of C3a And C5a
National Institutes of Health
R01 AI 057839 05/01/04 - 04/30/09 $232,541 / $1,213,711

Molecular Regulation Of Immune Complex Disease
National Institutes of Health
R01 AI 059305 12/15/04 - 11/30/09 $232,541 / $1,133,752

Current Year Direct **$2,558,923**

Funded Collaborative Efforts

Chougnet, C

Immunologic Dysfunction in Biliary Atresia
National Institutes of Health
J. Bezerra 08/15/03 - 01/31/13 8.5 %

Total $2,611,548