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RESEARCH CONFERENCES

ESF-EMBO Research Conference

B Cells and Protection: Back to Basics

Hotel Eden Roc, Sant Feliu de Guixols (Costa Brava) • Spain
12-17 June 2010

Chair: **Rita Carsetti,** Bambino Gesù Children's Hospital, Rome, IT
Co-Chair: **Deborah K. Dunn-Walters,** King's College London School of Medicine, UK

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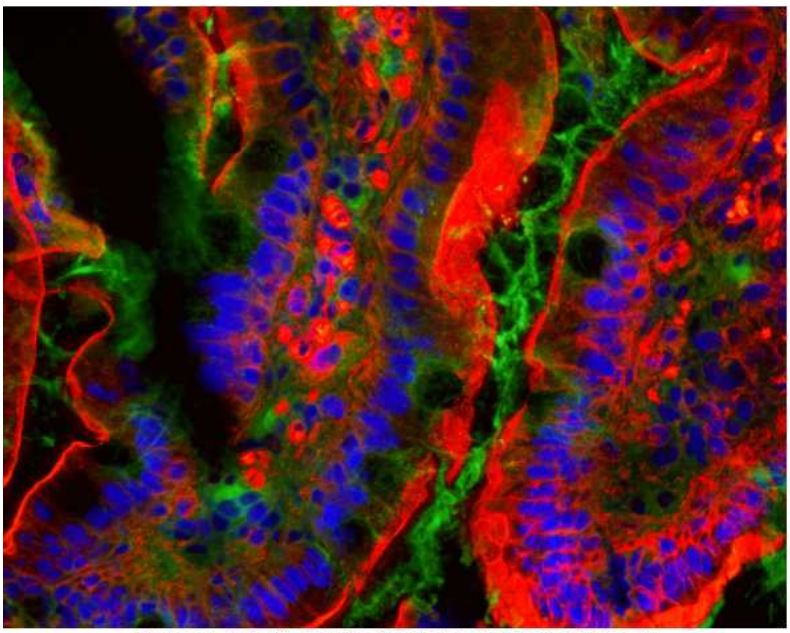
Highlights & Scientific Report



Source: R. Carsetti, Bambino Gesu Children Hospital, Rome, IT

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Conference Highlights

Please provide a brief summary of the conference and its highlights in non-specialist terms (especially for highly technical subjects) for communication and publicity purposes. (ca. 400-500 words)

The B cells and protection conference gathered together researchers and students coming from different scientific backgrounds and work experiences.
The main function of B cells is the generation of highly specific responses to a defined antigen after infection or vaccination. The idea that B cells are only important to generate high specific antibody responses is slowly changing into a more global view. In fact, B cells can play different roles in different tissues thus exerting crucial task in maintaining the equilibrium with the external world.

Bacteria are, and have always been, the dominant form of life on Earth. For multicellular organisms, that established millions of years after bacteria, co-existence and survival meant the development of multi-step barriers and the confinement of bacteria to special niches such as the lining surfaces of the mucosa. These sites of close contact represent places of active exchanges where perfect symbiotic interaction between microbiota and the organism have to be maintained. Because this equilibrium has been established very early in time, common tools are shared by non-related species often sitting very far in terms of evolution and complexicity.
One simple example comes from the Drosophila (commonly known as fruit fly), which we learn from the conference, has 15 species of bacteria in their gut, these are absolutely required for the survival and growth of the young larvae because they set-up the expression levels of receptors involved in growth.
In fact, it was in this model, that in the beginning of the 90's, the Toll gene and toll receptors (TLR) have been identified for the first time. TLR ligation, in fruit flies, plays an indispensable role on the protection against fungal infection in adults and on the embryonic development. With the discovery of TLRs started a new era on the field of innate immunity that soon extended to novel research topics also on acquired immunity.
TLRs represent innate receptors that bind repetitive structures called pathogen associated molecular patterns, but also endogenous ligands released during stress and/or necrosis, processes defined as sterile infection.
B cells "see" pathogens and react to them not only through their unique B cell receptor, but also using the more promiscuous and evolutionary ancient receptor TLR. Therefore, "antique" signalling pathways meet and integrate with the signals originated from the BCR and consequently new strategies can now be developed for vaccination and therapy.

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Scientific Report

Executive Summary

(2 pages max)

We are born 100% humans and we die 90% bacteria, meaning that after birth in each one's genetic patrimony 90% is of bacterial origin. Thus microbiota represents an indispensable component in the homeostasis of each single individual. Few pathogenic and many commensal microbes, that live "in and on" our body, help to develop the basis for the construction and maintenance of a healthy functional immune system.
For a long time, when talking of B cells, we have only considered relevant their highly specific response to immunization. Less then 10 years ago pioneer work by the group of Zinkernagel has pointed out the importance of natural antibodies. Most of the natural antibodies are generated by plasma cells differentiated from particular B cell subsets, such as B-1a B cells in the mouse and the so called IgM memory in humans, in an BCR independent way. Only recently we have started to understand the role of the innate receptors, such as TLRs, in the acquired immune response. On one side TLRs can be used to potentiate immune responses, by adding new pre-identified adjuvants to vaccination protocols. On the other hand they can be the targets for synthetic inhibitory molecules aiming the reduction of inflammation, for example in chronic sterile infections.

Until, recently the prevailing view that lymphocyte development and maturation are driven by DNA transcription factors, which are the dominant force in gene expression, is changing. We are passing from a DNA centric view to a more dynamic picture rolled by RNA handling mechanisms. Alternative splicing and differential polyadenylation appear to be more widespread than initially thought. Changes in mRNA decay rates affect the abundance of transcripts and this mechanism contributes significantly to gene expression. Mechanisms controlling the intracellular localization of mRNA and association with translating ribosomes are also important. Thus, gene expression is regulated through the coordination of transcriptional and post-transcriptional mechanisms leading to a more "RNA centric" view of gene expression. Currently identified the microRNAs (miRNAs) are a class of endogenous, non-coding regulatory RNAs that control gene regulation by guiding silencing protein complexes to mRNA in a sequence-dependent manner. The discovery of micro-RNA has taught us that one gene can be many genes and that this is important not only in tumorigenesis, but also in normal development. Also B-cell biology gained new levels of complexity by the discovery of microRNAs.

Scientific Content of the Conference

(1 page min.)

- *Summary of the conference sessions focusing on the scientific highlights*
- *Assessment of the results and their potential impact on future research or applications*

Microbiota represents an indispensable component in the homeostasis of each single individual. From the simple example of the Drosophila (commonly known as fruit fly), which we learned in the conference, that needs to be colonized by certain species of bacteria in their gut to survive and grow as young larvae (Francois Leulier), to mammals in which flora of the gastrointestinal tract promotes the maturation and maintenance of a functional immune system. An altered composition of the gut microflora as been correlated to increased incidence of chronic inflammation, autoimmune diseases, allergy and asthma. Several clinical studies indicated that a reduced presence of lactobacilli or bifidobacteria in the early intestinal microbiota of atopic children precedes the occurrence of allergic diseases. In fact, in mouse we learned that neonatal mono-colonization of germ-free animals with the Lactobacillus plantarum strain producing the major birch pollen allergen Bet v 1 attenuates the development of birch pollen allergy later in life. The

mechanisms involve a shift towards a non allergic Th1 phenotype accompanied by increased regulatory responses (Hana Kozakova).
Moreover, colonization of germ-free mice with pre-defined mix of intestinal commensal species results in the compartmentalized expansion, activation, and de novo generation of mucosal Treg cells specifically in the colon lamina propria (cLP). Induction of Treg cells is required for intestinal CD4+ T cell homeostasis and their efficient activation depends on the Toll-like receptor (TLR) adaptor molecules MyD88 and Ticam-1 (Andrew Macpherson).
Commensal induction of an intestinal cell response is therefore an important intrinsic mechanism for the establishment of immune homeostasis after colonization that lays the foundation upon which all subsequent immune responses can be build including the formation of the spleen (M. Manuela Rosado).
Mucosal antibody responses are another component of the equilibrium with the intestinal flora. It has been suggested that imbalance in the IgA+ and IgG+ intestinal B cell repertoire could be associated with the development of autoimmune diseases such as inflammatory bowel diseases (IBD) and Crohn's. Despite this, little was known about the antibody specificity of human intestinal plasmablasts. Here, we learned that approximately 25% of intestinal IgA and IgG plasmablast antibodies are polyreactive; the majority are antigen-specific. Antigen specificity covered both enteropathogenic microbes but also commensal microbes and self antigens. Regardless of their reactivity, all intestinal antibodies are somatically mutated and show signs of antigen-mediated selection, suggesting that they result from antigen-specific B cell responses (Hedda Wardemann).
These responses occur mostly in the Peyer's patches. Peyer's patches are like active and stable germinal centers where class-switch to IgA occurs, in fact we can find an increase of excision circles that are the end products of recombination. IgA pre-plasma cells can migrate from one Peyer's patches to another allowing the spreading of a given specificity though all the intestinal mucosa. Moreover these cells can re-enter the germinal center and increase their affinity by participating in another round of hypermutation and selection (Nils Lycke). In fact, the human gut is rich in AID expression, an enzyme required for both class-switching and somatic hypermutation reactions. Because in the intestine, from a single precursor we can obtain 10^{12} cells and for one heavy chain (V_H) mutation two cell divisions, virtually all the plasma cell repertoire in the intestine can be hypermutated (Jo Spencer).
We also learned that antimicrobial peptides, a-defensins, are important effectors of innate immunity shared by plants and animals. In mammals a-defensins contribute mostly to host defence against enteric pathogens, for example to segmented filamentous bacteria (SFB). SFBs are an important part of the indigenous flora, at present these bacteria cannot be grown in vitro but their presence and equilibrium in the intestine accounts for the maintenance of IL17 – producing lamina propria T cells (Nico Bos).
Another component of the innate immune response are the plasmocytoid dendritc cells (PDC), present also on the intestinal mucosa. They derive from unique bone marrow macrophage-dendritic cell precursors (MDP). The bone marrow-DC cluster is part of the perivascular system and can be identified by $MHCII^{high}$ $CD11c^{high}$ expression (Stefan Jung).

Sterile inflammation occurs when tissues are injured in the absence of infection. Is characterized by the accumulation of innate immune effector cells, namely neutrophils, within the affected tissue in reaction to necrotic cells. Such responses are classically considered homeostatic "wound healing" reactions to tissue injury, in which neutrophils clear debris by the phagocytosis and generate neutrophil extracellular traps (NETs) upon stimulation by platelets.
Sterile inflammation proceeds in a multi-step reaction that has been studied using a model of liver injury. Adenosine triphosphate released from necrotic cells activates the Nlrp3 inflammasome to generate an inflammatory microenvironment, rich in MIP that recruits circulating neutrophils via

CXCR2 to adhere within liver sinusoids. The generation of this intravascular chemokine gradient directs neutrophil migration through healthy tissue toward foci of damage. Dynamic in vivo imaging revealed a multistep hierarchy of directional cues that guide neutrophil localization to sites of sterile inflammation where they form NETs
Because neutrophils possess a vast arsenal of hydrolytic, oxidative, and pore-forming molecules capable of causing profound collateral tissue destruction, sterile inflammatory stimuli may contribute to the immunopathology observed in many diseases, including ischemic injuries/infarction, trauma, autoimmunity, drug-induced liver injury, and others (Craig Jenne).

Cells of the immune system are generated through a developmental cascade that begins in haematopoietic stem cells. During this process, gene expression patterns are programmed in a series of stages that bring about the restriction of cell potential, ultimately leading to the formation of specialized innate immune cells and mature lymphocytes that express antigen receptors. These events involve the regulation of both gene expression and DNA recombination, mainly through the control of chromatin accessibility. New technologies have supplied tremendous amount of information on chromatin and the nuclear machinery that affects protein–DNA structure including histone modification, nucleosome positioning, DNA methylation, replication timing and nuclear localization. All these complex multistep process are controlled and maintained by epigenetic mechanisms. The overall effect of the cis-acting epigenetic markers is to stabilize the effects of developmentally controlled trans-acting factors, and this, in turn, determines the potential of a cell and its progression through the differentiation process. Stem cells are already primed with specific sets of epigenetic marks that drive subsequent differentiation events. A similar epigenetic pre-marking system also exists for preparing genes for recombination at the immune receptor loci (Yhudit Bergman). Even though B-lymphocyte development is one of the best understood models for cell differentiation in the hematopoetic system, recent advances in cell sorting and functional genomics has increased this understanding further. Early lymphoid primed multipotent progenitor cells (LMPPs) express low levels of lymphoid restricted transcripts. The expression of these genes becomes more pronounced when cells enter the FLT-3/IL-7 receptor positive common lymphoid progenitor (CLP) stage but the expression of B-lineage specific genes is limited to a B-cell restricted Ly6D surface positive subpopulation of the CLP compartment. Inside the heterogeneity of the CLPs it has been found a point of no return in B-cell development in which Ly6D positive cells express the transcription factors EBF-1, PAX-5 and E47 (Mikael Sigvardsson).
The chromatin regulator Aiolos and the transcriptional coactivator OBF-1 are also implicated in B cell maturation and activation. In mice the lack of both factors results in a block at the transition between pre-B and immature B cells. The numbers of immature B cells are reduced, small pre-BII cells are increased and a significant impairment in immunoglobulin light chain DNA rearrangement is observed. The components of the pre-BCR, such as the surrogate light chain genes fail to be efficiently silenced and early pre-B cells are not able to express a viable BCR to receive the cell survival signals necessary to progress in their development (Ing-lill Martensson).

One of the key factors regulating cell survival and thus B cell development is phospholipase C (PLC)γ2. PLCγ2 deficient mice show reduced levels of the pro-survival protein Bcl-2 and a defect in the development of transitional T3 and marginal zone (MZ) B cells. Restriction on the BCR repertoire by the insertion of a rearranged BCR results in a reduction in the number of IgM-positive B cells and a paucity of IgD-expressing cells in the spleen reflecting the low survival capacity of these cells (Martin Turner).
miRNA are small molecules of RNA that can regulate lymphocyte development and function. One of the approaches used to understand the control of lymphocyte function by miRNAs has been the

generation of animal models in which global miRNA maturation is blocked through the deletion of Dicer endonuclease. Dicer plays a crucial role in miRNA biogenesis by cleaving pre-miRNAs to generate a double strand RNA duplex that contains the mature miRNA. Dicer deletion in the mouse germline has a lethal phenotype, but conditional Dicer alleles have allowed addressing its role in specific cell lineages. Here we learned that disruption of Dicer with mb-1Cre leads to the almost complete block at the pro-pre-B cell transition due to massive apoptosis caused by the aberrant regulation of Bim protein. If Dicer is depleted at later B cell stage, the CD19-Cre mouse strain, there is an inbalance generation of marginal zone (MZ) versus follicular (FO) B cell spleen subsets. This differentiation phenotype is accompanied by a skewed BCR repertoire with the generation of self-reactive antibodies and autoimmunity. miR-185 is the critical player in this phenotype through the regulation of BCR signalling by Btk (Almudena Ramiro).

Moving to more mature phases of the B cell development we arrive to memory B cells and the terminal differentiated antibody secreting cells.
It is known that old people are more susceptible to morbidity and mortality from infectious diseases, particularly from pulmonary diseases such as pneumococcal pneumonia where vaccines do not provide efficient protection as in younger populations. Here we found that not only the B-cell repertoire in the old is reduced but also that serum IgM and IgA pneumococcal responses are significantly impaired in the elderly, with no difference in IgG levels. Analysis of the spectratypes before and 28 days after vaccination indicates that the baseline repertoire in the older group was comprised of larger CDR3 regions than in the younger group resulting in reduced diversity. Hydrophilicity and/or small size of the IGH CDR3 appear to be important in pneumococcal responses. IGHM spectratype analysis seems to be the most promising in terms of its predictive ability for vaccine responses (Deborah Dunn-Walters).
Lifelong antibody responses to vaccination require maintenance of the plasma cell niche in the bone marrow. Persistence of secreting cells at this site depends on the availability of survival factors such as APRIL (Michael Cancro). Because APRIL is increased in a variety of autoimmune disorders, this factor contributes for the survival of self-reactive secreting cells and autoantibodies (Heipe Falk).

Forward Look

(1 page min.)

- *Assessment of the results*
- *Contribution to the future direction of the field – identification of issues in the 5-10 years & timeframe*
- *Identification of emerging topics*

The program of the meeting gathered together scientists coming from several fields of expertise and students from various origins.
It emerged from the presentations and discussions that the classification and the roles of host and pathogen have to be redefined. The indigenous flora of the gastrointestinal tract is set up soon after birth, the type and amount of bacterial exposure revelled to be determinate for the maturation of the immune system, “imprinting” the host with a future of health or disease. Thus, microbiota or better the microbiome, that is the totality of microbes, their genomes, and environmental interactions in a particular environment should be analysed as part of the human genome, because of their influence on human physiology.
Because our genetic background is not only genes but also complex RNA regulatory elements, defining miRNA expression profiles in specific cell lineages is the most useful tool to identify miRNAs potentially relevant to their development or function. A number of analyses of miRNA profiling in particular lineages of the immune system have been done using various techniques but all the information still needs to be confirmed by matching these miRNAs to their cognate transcripts in vivo. The identification of cell specific microRNAs and the targets for their regulatory function can open novel therapeutic tools.

The future of B cell immunology has never looked so exciting, with the discovery of many new functions of B cells. Novel technologies and applications of tools from disparate disciplines combine with emerging biological information to ensure that B cell immunity is at the forefront of biological / health research.

Identification of emerging topics:
Microbiome
MicroRNA profiles
What determines long lasting immunological memory?

▪ **Is there a need for a foresight-type initiative?**

We believe that a meeting promoting topics such as the ones of the former conference or on topics addressing interactions between bacteria (pathogen and commensals) and B cell physiology is necessary in the European context. Moreover, such conferences are always important spots to discuss new findings and points of view in a setting where senior and junior scientists live in close contact and exchange knowledge and ideas in order to create a fertile humus for a competitive European science.

Atmosphere and Infrastructure

▪ *The reaction of the participants to the location and the organization, including networking, and any other relevant comments*

The friendly ambience present during section discussions resulted in the generation of new ideas, working hypothesis and contacts for future collaborations among European scientists.
The infrastructure was appropriate, provided a good service and the staff was available and affable.

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Date & Author: 30 June 2011 on behalf of Rita Carsetti, Maria Manuela Rosado