Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Immunology

Editorial overview: Lymphocyte activation and effector functions

Claude-Agnès Reynaud and Stuart Tangye

Current Opinion in Immunology 2014, **28**:v–vii

For a complete overview see the Issue

Available online 18th April 2014

0952-7915/$ – see front matter,

http://dx.doi.org/10.1016/j.coi.2014.04.001

Claude-Agnès Reynaud
Inserm U783, Faculté de Medecine Necker-Enfants Malades, Paris Descartes Medical School, Paris, France
e-mail: claude-agnes.reynaud@inserm.fr

Claude-Agnès Reynaud is director of research at Institut Necker-Enfants Malades, Paris Descartes Medical School, and head of the group 'Development of the immune system'. She has a long-standing interest in molecular mechanisms of Ig gene repertoire formation, and more recently extended the focus of her studies to the functional diversity of B-cell memory/effector subsets, both in the mouse and in humans.

Stuart Tangye
Garvan Institute of Medical Research, Darlinghurst, Australia
e-mail: s.tangye@garvan.org.au

Stuart Tangye is an associate professor, principal research fellow at the Garvan Institute of Medical Research (Sydney, Australia) and head of the 'Immunology and Immunodeficiency' lab. He undertook post-doctoral training at the DNAX Research Institute of Molecular and Cellular Biology (Palo Alto, California), where he investigated signaling, activation and differentiation of human T cells, NK cells and B cells. His research is focussed on studying human lymphocyte subsets, differentiation and immunological memory, and how these processes are compromised in individuals with monogenic immunodeficiencies.

Lymphocyte activation requires the integration of signals provided by the extracellular environment in the form of cell–cell interactions and soluble factors, mainly cytokines. Engagement of specific receptors by these ligands trigger myriad signaling pathways that induce expression and/or activation of transcription factors that ultimately function to modulate gene expression, thereby imprinting the cell with a particular effector cell fate. The delivery of activating signals needs to be counterbalanced by regulatory pathways, in order to maintain immune homeostasis. The importance of such immune regulation is evidenced by the development of numerous immunological diseases — including autoimmunity, allergy, immune deficiency and malignancy — when this balance is impaired.

The regulatory control of lymphocyte activation, and in particular the signals that trigger a T-dependent immune response, appear in all immunology textbooks. However, many steps, including survival cues and selection forces that shape the efficacy and persistence of the immune reaction, are still elusive and require new approaches to allow us to understand how autoreactive, allergic or infectious processes can escape immune control. This volume puts together nine reports discussing recent advances coming from horizons as diverse as systems biology, imaging technologies, establishment of new mouse reporter lines or studies of human primary immunodeficiencies: a majority of them concern selection events occurring during B cell activation, but their focus also extend to broader issues on the activation of subsets of T helper cells.

Three reviews discuss key events occurring in the germinal center (GC) environment. Gabriel Victora presents major advances, contributed predominantly by intravital microscopy, in the understanding of the selection steps leading to affinity maturation within this unique microenvironment. These advances concern notably the identification of specific transcription profiles for the dark zone and light zone compartments of a GC, as well as the role of T follicular helper (T_{FH}) cell-derived signals in controlling the shuttling between these two zones. As highlighted by the author, intravital microscopy has thus recently provided experimental evidences for the long-standing proposition of a cyclic re-entry of GC B cells in the dark zone as a key control step in the selection and expansion of high affinity B-cell clones.

Many other levels of regulation are exerted during the major transcriptional reprogramming that takes place in this microenvironment for both B and T_{FH} cells. One key regulatory mechanism is the control of gene expression by micro RNAs (miRNAs). Dirk Baumjohann and Mark Ansel review the mandatory role that the miR-17–92 cluster (encoding 6 miRNAs) plays in the differentiation of T_{FH} cells. miRNAs appear to regulate multiple steps, from pro-apoptotic factors (Bim) or tumor suppressors (PTEN) to

transcription factors governing alternative differentiation programs (e.g. RORα), thus enforcing T_{FH} identity. Several miRNAs also regulate distinct steps of the GC B cell reaction, both negatively or positively, as established for miR155 that promotes the GC response but downregulates activation-induced cytidine deaminase (AID), the key factor triggering affinity maturation and class switch recombination. Not unexpectedly, dysregulation of miRNA expression, and notably of the miR-17–92 cluster, appears as a major contributor to lymphomagenesis.

Pathogenic autoantibodies frequently display high level of somatic mutations, suggesting that autoreactive B cells emerge during the second wave of diversification occurring during the GC reaction. Robert Brink discusses new insights provided by a mouse model in which an Ig transgene frequently acquires high affinity against a self-protein during the immune response, and which showed that the emergence of autoreactive B-cell clones depends on systemic versus organ-specific expression of the self-protein. Anti-DNA antibodies might represent a specific configuration in which co-stimulation of BCR and TLR pathways favor the escape of reactive B cell clones from immune tolerance in the setting of persistent stimulations. Robert Brink further reviews recent studies of human autoimmune diseases, in which mutated pathogenic antibodies have been reverted to their germ-line configuration to identify the original driving force. Interestingly, such analyses have revealed a broad diversity of situations, from authentic molecular mimicry as in Graves' disease to more fundamental breaches of tolerance, as for anti-GM-CSF antibodies linked with idiopathic pulmonary alveolar proteinosis.

T_{FH} cells are a more recently described subset of $CD4^+$ T cells. T_{FH} cells are characterized by expression of the transcriptional repressor Bcl6, their localization within GCs, and their key role in providing cognate help to GC B cells, allowing the formation of high affinity memory B cells and plasma cells. However, whether T_{FH} cells represent a stable lineage of effector $CD4^+$ T cells remains an open question. Hai Qi and colleagues summarize recent advances and questions in this fast moving field, notably concerning the early induction of Bcl6 in pre-GC T_{FH} cells and its role in promoting follicular localization by — possibly indirectly — promoting CXCR5 and ICOS expression. It is also unclear at which step lineage commitment effectively takes place, from the first activation of a naive T cell by dendritic cells to its migration to the T–B border, and then through the follicular region to its final interaction with GC B cells. At the other end of the GC reaction, the identification of T_{FH}-like cells in blood and the similar requirement by central memory T cells for bcl6, CXCR5 and ICOS expression questions the potential of T_{FH} cells to contribute to central memory, and the stability of this lineage in a recall response.

The discovery of IgE was made more than forty years ago, and it is surprising how little we know regarding the physiology of the IgE response. Recent advances in the field have originated from novel dedicated mouse reporter lines, allowing the sensitive assessment of IgE^+ B cells and plasma cells via their intrinsic fluorescent labeling. Chris Allen and colleagues summarize results obtained in three independent IgE reporter models established in mice, which all conclude that a specific survival defect during the GC reaction, and an intrinsic bias toward a short-lived plasma cell fate, restrict IgE production *in vivo*. Understanding the specificity of signals transduced by the Ig ε heavy chain that account for this biased differentiation profile is the next challenge in this new area, as well as assessment of IgE^+ cells in conditions of allergic diseases. Together, these studies may provide the foundation for the development of novel therapeutics for the treatment of IgE-mediated diseases, such as allergy.

Whether regulatory B cells represent a distinct lineage or an activation state remains a contentious debated issue, with their precise phenotype and the approaches employed to identify them differing between various investigators. The review by Simon Fillatreau and colleagues suggests that such regulatory function may in fact be the attribute of differentiating plasma cells, which would secrete a transient wave of cytokines modulating the magnitude or profile of an acute immune response. IL-10, IL-35, GM-CSF, IL-17 and TNFα/iNOS secretion have indeed been linked to distinct, non-overlapping B-cell subsets at various stages of activation and/or differentiation toward the plasma cell lineage in mice. This challenging vision of B cells as active contributors of the cytokine milieu, with either an anti-inflammatory or pro-inflammatory role, provides a new framework for understanding autoimmune or infectious pathologies.

The cognate and non-cognate help provided to B cells was recently extended to an unconventional population of the T-cell family — invariant NK-T cells — which appear as efficient and complex contributors to the generation of protective humoral immune responses for both non-protein or protein antigens. Beyond the well-known capacity of iNKT to recognize the α-Galactosylceramide (αGalCer) glycolipid presented by the invariant MHC class I molecule CD1d, Elizabeth Leadbetter and colleagues develop the interesting notion that iNKT cells can provide both innate/cognate and adaptive/non-cognate help to B cells, cognate and non-cognate being used from the 'point of view' of the iNKT cell. Cognate help will occur, for example, with a hapten coupled to αGalCer triggering direct provision of activating cytokines to the glycolipid Ag-presenting B cell, while non-cognate help, triggered by glycolipid plus protein injection, will be driven by dendritic cells activating massive cytokine secretion from iNKT and enhancement of a cognate $CD4^+$ T cell response. Several recent reports further

described that nanoparticles containing glycolipids together with protein or even polysaccharide antigens can elicit robust help from iNKT leading to a long-lasting protective response, and thus appear as promising tools for effective vaccine development.

The use of systems biology is a powerful approach to deciphering the specific requirements for distinct lineage commitment, and detailed analysis of T_H17 cells represent probably the most remarkable achievements in this field. Anneli Peters and Nir Yosef describe transcriptional regulatory networks that have emerged from such recent studies. First, from studies of transcriptional signature and chromatin occupancy by defined factors, a picture emerged in which chromatin accessibility is initially provided by coordinate binding of IRF4 and BATF to the so-called AP-1-IRF composite elements (AICE sites), allowing 'master regulators' such as RORγt to bind these pre-established sites. A second series of reports further interrogate data from gene expression profiling obtained from a time series or genetic perturbations (including through new nanowire siRNA delivery) together with novel computational methods, to describe a complex network of positive (including new factors like MINA) or negative (like FosL2) regulators that concur to the stability of the T_H17 profile. Such a network of interaction may obviously reveal potential therapeutic targets among the new regulators or effectors uncovered.

A wider picture of the different actors of the immune response is provided through a review by Stuart Tangye and colleagues of the human primary immunodeficiency autosomal dominant hyper-IgE syndrome, which is caused by dominant negative loss-of function mutations in *STAT3*, as well as related conditions resulting from complete inactivation of various cytokine and cytokine receptors that signal through this hub for lymphocyte effector function. The major impact is on T_H17 and T_{FH} subsets, and a marked reduction in memory B cell formation due to both compromised IL-21 provision and IL-21R signal transduction. Interestingly, some of the observations derived from these clinical cases contrast with those made in mice (notably concerning the function of Tregs or T_H2), highlighting the unique contribution of such studies to the understanding of the human immune system. While these observations easily allow the correlation of susceptibility to fungal infections and impaired Ab responses observed in this syndrome to the loss of T_H17 function and memory B cells, they are yet to provide a clear explanation for the cause of hyper-IgE, which is clearly an Ig isotype with unique regulation. As highlighted by the authors, the impact of STAT3 deficiency on other partners of the immune response (NK, neutrophils) remains to be studied.

Together, this collection of reviews provides novel insights into the cellular, biochemical and molecular requirements for the initiation, progression, fine-tuning and attenuation of cell-mediated and humoral immune responses. These serve as a reminder of the significant advances that have been made in recent times that facilitated break-through findings in age-old and contemporary questions relating to immune regulation. Hopefully, the next wave of discoveries will facilitate greater translation to our understanding of human disease and allow the development of novel therapies for a panoply of immune dyscrasias ranging from immunodeficiency and autoimmunity, to allergy and cancer.