VIEWPOINT

Highlights of 10 years of immunology in *Nature Reviews Immunology*

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Abstract | As *Nature Reviews Immunology* reaches its 10th anniversary, the authors of one of the top-cited articles from each year take a trip down memory lane. We've asked them to look back on the state of research at the time their Review was published, to consider why the article has had the impact it has and to discuss the future directions of their field. This Viewpoint article provides an interesting snapshot of some of the fundamental advances in immunology over the past 10 years. Highlights include our improved understanding of Toll-like receptor signalling, and of immune regulation mediated by regulatory T cells, indoleamine 2,3-dioxygenase, myeloid-derived suppressor cells and interleukin-10. The complexities in the development and heterogeneity of macrophages, dendritic cells and T helper cells continue to engage immunologists, as do the immune processes involved in diseases such as atherosclerosis. We look forward to what the next 10 years of immunology research may bring.

2001 TLRs: a decade of excitement

Ruslan Medzhitov. The discovery of Toll-like receptors (TLRs) has provided fundamental insights into the biology of the innate immune system. The description of TLR functions supported the key tenets of Janeway's pattern recognition theory, which posited that microbial recognition by dedicated receptors controls activation of the adaptive immune system. In 2001, when my Review was published[1], the TLR field was just starting to explode, with numerous discoveries being reported on a monthly basis. It was already becoming clear that TLRs are the most potent inducers of the inflammatory response, that most microbial preparations or purified molecules with immunostimulatory activities are sensed by TLRs, and that TLR ligands are potent activators of dendritic cell (DC) maturation. In addition, TLRs provided the first clear example that pattern recognition receptors of the innate immune system can control the activation of adaptive immune responses. These early studies also revealed that TLRs control some, but not all, adaptive immune responses, indicating that additional pathways must exist.

Since these early days of TLR biology, much progress has been made in the field. The specificities of most TLRs for their microbial ligands have been elucidated. The first few structures of TLRs complexed with their ligands have provided spectacular insights into the mechanisms of pattern recognition. They revealed how the shared molecular patterns of conserved bacterial lipids are recognized by TLRs, either directly, or through the accessory protein MD2. Completely unexpectedly, TLR7 and TLR9 were found to undergo proteolytic processing in their ectodomain to become competent for ligand recognition. These findings were surprising, as they indicated that large portions of the ectodomains of these TLRs are dispensable for ligand binding.

Although major components of the signalling pathways downstream of TLRs have been fairly well characterized, the exact mechanisms by which TLRs induce gene expression remain largely unknown. Indeed, these signalling pathways have turned out to be far more complex than was expected 10 years ago (FIG. 1) and to involve a high degree of cross-regulation that is only starting to be elucidated. Particularly fascinating are the cell biological themes that recur in TLR signalling. For example, TLRs have been found to induce distinct signalling pathways from different cellular compartments, highlighting the role of TLR trafficking in signal transduction. Equally exciting are *in vivo* findings that implicate TLRs in the initiation of sterile inflammation. Here, TLRs may play a protective role by orchestrating tissue repair, but this very function may also contribute to tumorigenesis and other pathologies.

The role of TLRs in pathogen recognition is now well documented, although it is not yet fully understood. Some questions that existed in the TLR field 10 years ago remain unanswered or, at best, poorly characterized. For example, what is the full spectrum of endogenous TLR ligands? Do endogenous TLR ligands elicit the same responses as pathogen-derived ligands? What is the biological significance of sterile inflammation induced by endogenous TLR ligands? What are the functions of TLRs outside of the immune system and host defence? Several examples that implicate TLRs in the control of metabolism and tissue repair are known, but the mechanisms involved are not yet defined. Do TLRs distinguish between commensal and pathogenic microorganisms and, if so, how? How do TLRs functionally interact with other pattern recognition receptors, such as retinoic acid-inducible gene I (RIG-I), melanoma differentiation-associated gene 5 (MDA5) and the dectin family of lectin-like receptors? Several new families of receptors involved in pathogen recognition have been characterized over the past few years, and several more types of innate immune sensor probably exist that have yet to be described. Given that these receptor families have very different properties, it would be interesting

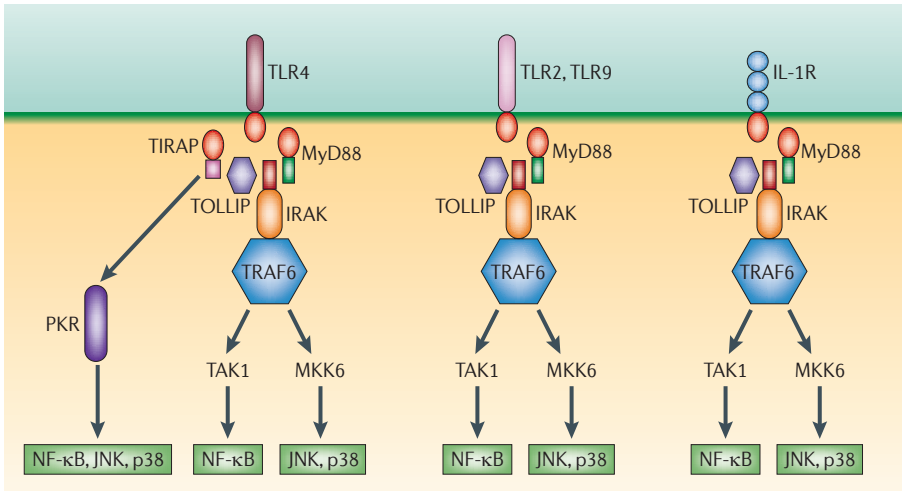

Figure 1 | **Toll signalling pathways.** The Toll-like receptor (TLR) and interleukin-1 receptor (IL-1R)-family members share several signalling components, including the adaptor MyD88, Toll-interacting protein (TOLLIP), the protein kinase IRAK (IL-1R-associated kinase) and TRAF6 (TNF receptor-associated factor 6). TRAF6 can activate nuclear factor-κB (NF-κB) through TAK1 (TGF-β-activated kinase), and JNK (c-Jun N-terminal kinase) and p38 MAP kinases through MKK6 (mitogen-activated protein kinase kinase 6). TLR4 signals through another adaptor in addition to MyD88–TIRAP (Toll/interleukin-1 (IL-1) receptor domain-containing adaptor protein), which activates MyD88-independent signalling downstream of TLR4. The protein kinase PKR functions downstream of TIRAP, but its importance in this pathway has not yet been established. Image is reproduced, with permission, from REF. 1 © (2001) Macmillan Publishers Ltd. All rights reserved.

to determine in future studies whether they have equivalent and autonomous roles in host defence, or whether they have to operate in specific combinations.

Fashions in science come and go. The most important discoveries, however, are made before and after the subject is in fashion. Perhaps TLRs still keep some of their best secrets.

2002 T_{Reg} cells: many unanswered questions remain

Ethan M. Shevach. I am delighted that my article in *Nature Reviews Immunology* entitled 'CD4$^+$CD25$^+$ suppressor T cells: more questions than answers'[2] was one of the top-cited articles of 2002. It was published in the right place at the right time. At the beginning of the 21st century, the concept that CD4$^+$CD25$^+$ T cells[3] were a population of dedicated regulatory (suppressor!) T cells (T_{Reg} cells) was beginning to be accepted by the immunological community, who had finally left behind the unpleasant memories of the suppressor T cell field of the 1970s. Interest in the field increased exponentially in 2001, when a similar population of cells was identified in human peripheral blood[4]. The few remaining non-believers were rapidly converted in 2003 by the identification of the transcription factor forkhead box P3 (FOXP3) as both the phenotypic and functional marker of this cell population[5].

My intent in writing the Review was to highlight important questions that needed to be answered by future studies. Rather than relive the past, I emphasize here the future, as T_{Reg} cells have already entered the clinic[6]. What I find most remarkable is that many of the major questions I raised in 2002 remain unanswered today.

One, what is a T_{Reg} cell? Multiple subsets of FOXP3$^-$ T cells have been shown to have regulatory activity. Are any of these populations of biological importance or therapeutic interest? We still lack a surface marker that definitively identifies functional FOXP3$^+$ T_{Reg} cells. In addition, it is now established that FOXP3$^+$ T_{Reg} cells can be the product of thymic development (called natural T_{Reg} cells) or can be generated from conventional CD4$^+$ T cells in peripheral sites (called induced T_{Reg} cells)[7]. Natural and induced T_{Reg} cells have distinct T cell receptor repertoires, potentially use different regulatory mechanisms, have different survival and self-replication properties, and have different levels of stability of FOXP3 expression. There is a critical need for a cell surface marker that can distinguish between natural and induced T_{Reg} cells, so that their numbers and functions can be monitored and characterized. Moreover, do T_{Reg} cells readily convert into effector T cells (plasticity) and are induced T_{Reg} cells more plastic than natural T_{Reg} cells? Which population of T_{Reg} cells should be used therapeutically?

Two, what are their mechanisms of action? No major mechanism of action for T_{Reg} cells has been discovered, and the prevailing view is that FOXP3$^+$ T_{Reg} cells can select from a long list of contact-dependent, contact-independent and metabolic mechanisms to mediate their suppressive functions in a context-dependent manner[8]. A further complexity is the long list of cells of both the innate and adaptive immune systems (as well as non-haematopoietic cells) that can serve as targets for suppression[9]. How can we approach clinical manipulation of T_{Reg} cell function when we still have a poor understanding of the mechanisms used by T_{Reg} cells in any complex human disease?

Three, are T_{Reg} cells defective in autoimmune diseases? Why raise this question in view of the multiple reports demonstrating defective T_{Reg} cell function in every autoimmune disease? Put simply, if we really do not know how T_{Reg} cells function, how can we claim that their function is abnormal? A great reliance has been placed on 'standard' *in vitro* suppression assays, but these assays may have absolutely no *in vivo* relevance[9]. Would cellular therapy of autoimmune disease with *in vitro*-expanded patient-derived T_{Reg} cells be effective if these cells were truly defective? Pharmacological correction of the defective function would seem to be the more appropriate approach[10].

Hopefully, studies over the next 10 years will resolve these issues and the manipulation of T_{Reg} cell function will become a therapeutic reality.

2003 The IL-12 cytokine family

Giorgio Trinchieri. My 2003 Review entitled 'Interleukin-12 and the regulation of innate resistance and adaptive immunity'[11] appeared at an interesting critical time in the history of the interleukin-12 (IL-12) family of heterodimeric cytokines and of the regulation of the T helper (T_H) cell responses. My group described IL-12 in 1989 as a natural killer (NK) cell-activating and interferon-γ (IFNγ)-inducing factor (termed NKSF)[12], and we cloned the genes encoding its two chains in 1991 (REF. 13). A couple of years later, we[14] and Hsieh *et al.*[15] demonstrated that IL-12 has an instrumental role in the induction of T_H1 cell responses in humans and mice, respectively. A large number of studies in the years preceding our Review extensively characterized and dissected the functions of IL-12. These investigations established that IL-12 has a clear, important role in the expansion and maintenance of optimal T_H1 cell responses in infections, but that its role in the initiation of the response

is not unique, as T_H1 cell responses could also be observed in the complete absence of IL-12 (although possibly not as effectively as in its presence)[16]. The ability of antibodies specific for the p40 chain of IL-12 to inhibit the pathology in several models of autoimmune diseases was considered as evidence for the role of IL-12 and T_H1 cell responses in autoimmunity.

However, as described in my Review[11], two new members of the IL-12 family — IL-23 and IL-27 — were reported by a group at the DNAX Research Institute, California, USA, in 2000 and 2002, respectively[17,18]. The IL-23 heterodimer shares the p40 chain and a receptor chain with IL-12, whereas the IL-27 heterodimer has structural and sequence homologies with IL-12 but no shared components. Initially, it was thought that IL-23 and IL-27 had partially overlapping functions with IL-12, but the discovery that most of the inhibitory effect of IL-12p40-specific antibodies on autoimmunity was due to their ability to inhibit IL-23 dramatically revolutionized the field[19,20]. IL-23 was found to be one of the important factors for inducing and maintaining a T_H17 cell response, and the role of T_H1 and T_H17 cells in mediating autoimmunity was re-evaluated[20,21].

At the same time, the immune-activating functions of IL-27 had been re-evaluated, and the important role of IL-27 in the down-regulation of T cell responses (particularly those of T_H1 and T_H17 cells) and in the induction of IL-10 production by T cells was fully appreciated[21]. Given that my 2003 Review appeared at such a critical junction in the study of T cell immunity, and therefore at a time of renewed interest in this family of immune regulatory cytokines, it is of no surprise that it was highly read and cited.

2004 IDO: answering outstanding questions

Andrew L. Mellor and David H. Munn. Indoleamine 2,3-dioxygenase (IDO) is an intracellular enzyme that degrades indole compounds, including tryptophan. Before 1998, IDO was thought to participate in innate host defence against pathogens. But, in 1998, we proposed that IDO also has a role in immune regulation, as inhibiting IDO during pregnancy in mice allowed maternal T cells to attack allogeneic fetal tissues. By 2004, many studies had identified IDO as a component of the inflammatory response associated with infection, cancer and autoimmune and allergic diseases.

At the time of our 2004 Review[22], three key questions were unresolved: one, the role of IDO in immune regulation and the therapeutic potential of manipulating IDO activity; two, the origin and biological significance of 'IDO-competent DCs'; and three, the molecular mechanisms that explained IDO-mediated regulation of immunity.

Substantial progress on all three issues has been made in the last 7 years. IDO has been shown to control local innate immunity (inflammation) and to regulate antigen-specific adaptive immune responses in settings as diverse as mucosal tolerance, asthma, acquired tolerance to allografts, chronic infection and tumour-induced immunosuppression. In the case of cancer, numerous studies have identified IDO expression in patients with malignancy. Treating tumour-bearing mice with IDO inhibitors enhances tumour-specific immunity and synergizes with cytotoxic chemotherapy to improve treatment of established tumours[23,24], and IDO inhibitors have recently entered Phase II oncology trials.

In infectious disease, the classical view that IDO inhibits pathogen replication appears to apply in some settings. In other settings, pathogen-induced IDO may attenuate host T cell immune responses and facilitate the persistence of certain infections, such as HIV and tuberculosis[25,26]. Thus, depending on the pathogen, IDO may help or hinder antimicrobial immune responses, and this is an active area of research[27]. IDO expression is also a common feature of autoimmune, allergic and graft-versus-host diseases, and in these settings IDO inhibition markedly exacerbates disease severity.

In the case of transplantation, David Wilkes and colleagues[28] first showed that *IDO* gene transfer protected lung allografts, and similar approaches have been used to protect pancreatic islet, skin and corneal allografts. Of note, overexpression of IDO in transplanted lungs profoundly impairs the cytotoxic functions of host T cells that infiltrate the allografts[29]. Therefore, the common theme that permeates all of these studies is that IDO helps to establish acquired immunological unresponsiveness at sites of inflammation.

At the molecular level, IDO has been shown to promote immune regulation in two ways. First, it depletes tryptophan, and this activates the cellular stress response pathway via the kinase GCN2 (also known as EIF2AK4)[30]; and, second, it produces kynurenine metabolites, which bind to the aryl hydrocarbon receptor[31]. IDO can be induced by pro-inflammatory cytokines or by a 'reverse signalling' pathway via the interaction of CD80 and CD86 on DCs with cytotoxic T lymphocyte antigen 4 (CTLA4) expressed by T_{Reg} cells[32]. The induction of IDO activity in DCs (IDO-competent DCs) drives the differentiation of naive $CD4^+$ T cells into $FOXP3^+$ T_{Reg} cells and also induces the activation and regulatory function of functionally quiescent T_{Reg} cells[33,34]. IDO also appears to stabilize the regulatory phenotype of T_{Reg} cells and prevent T_{Reg} cell reprogramming into helper-like T cells[33]. Thus, IDO continues to emerge as a pivotal molecule in acquired immunological unresponsiveness that occurs at sites of inflammation in settings of clinical significance.

2005 Macrophage heterogeneity: are we (still) missing the point?

Siamon Gordon. The heterogeneity of monocytes, macrophages and closely related DCs and osteoclasts has been a recurring theme in the study of these specialized myeloid-derived phagocytes. They are found in haematopoietic compartments and the circulation, and are also present in large numbers in tissues, even in the absence of inflammation. They have a bewildering diversity of phenotype, a feature of both opportunity and frustration for immunologists. Earlier discussions of nomenclature gave rise to terms such as the reticuloendothelial system and mononuclear phagocyte system. But it wasn't until the advent of monoclonal antibodies specific for glycoproteins that appear to be restricted to these cell types (including F4/80 and CD68) that tissue variation in the morphology of macrophages (for example, in the liver, skin and brain) could be correlated with differences in the expression of these molecules[35].

Cells of this unitary lineage can be identified during development and, in adults, they occur in haematopoietic organs, in the blood (as monocytes) and in tissues (constitutively as resident macrophages and also as recruited inflammatory cells). However, it was difficult to distinguish between their differentiation, maturation and modulation (activation and deactivation). The neglected topic of population dynamics and precursor–product relationships was transformed by the study of Geissmann and Jung[36] using CX_3C-chemokine receptor 1 (CX_3CR1; also known as fractalkine receptor)-transgenic mice, which showed that monocyte subsets exist in mice and also provided an experimental tool to confirm and extend earlier studies by Ziegler-Heitbrock[37] on human monocytes.

Our Review in *Nature Reviews Immunology*[38] was therefore opportune and timely, but only the beginning of an ongoing flow of reviews on this subject. The topic continues to provoke controversy, as is evident from a recent attempt to clarify issues,

and a consensus has not been reached among several investigators[39]. Terms such as plasticity and polarization, and the so-called M1 and M2 phenotypes, are poorly defined and incompletely understood, especially as microarray and proteomic analyses accumulate.

Anniversaries — 30 years for F4/80 and 10 years for *Nature Reviews Immunology* — provide an opportunity to assess where we are now, as a pointer to the future. My personal view is that, as a field, we are to a great extent still missing the point. In order to understand the extent and significance of monocyte and macrophage heterogeneity we need to investigate the population dynamics and individual cell functions of the bulk of macrophages within the different compartments of the body *in situ*. Macrophages embedded in tissues are often difficult to isolate and the cells rapidly lose their special features once removed from their native microenvironment. Fluorescence-activated cell sorting (FACS) analysis is biased by ease of isolation; immunocytochemistry is qualitative; and *in situ* hybridization is cumbersome, as is laser capture microscopy. Intravital microscopy and transgenesis offer further experimental opportunities.

Macrophages are found in the absence of disease in all organs, but we know very little about the role of these cells in sites such as the brain (where they are termed microglia), gut, lung and elsewhere. Although monocytes and macrophages that are recruited during inflammation are better characterized than resident macrophages, there is still considerable ignorance regarding their origin, distribution, longevity and modulation by immune and non-immune stimuli. This applies to their complex roles in promoting or counteracting many disease processes, including infections (for example, tuberculosis, HIV/AIDS and malaria), as well as their roles in modified inflammatory pathologies such as malignancy, atherosclerosis and Alzheimer's disease.

Through their array of surface and intracellular receptors, monocytes and macrophages adapt readily to their microenvironment. Their versatile gene expression, protein synthesis and secretory capacity are legendary. It seems invidious to select only immune complexes[40] or a limited number of cytokines (such as IFNγ, IL-4, IL-13 and IL-10)[41,42] among many stimuli as inducers of characteristic signatures of gene expression and as regulators of macrophage functional diversity. Expression and activation of macrophage transcription factors, as well as epigenetic and translational controls, are still poorly defined.

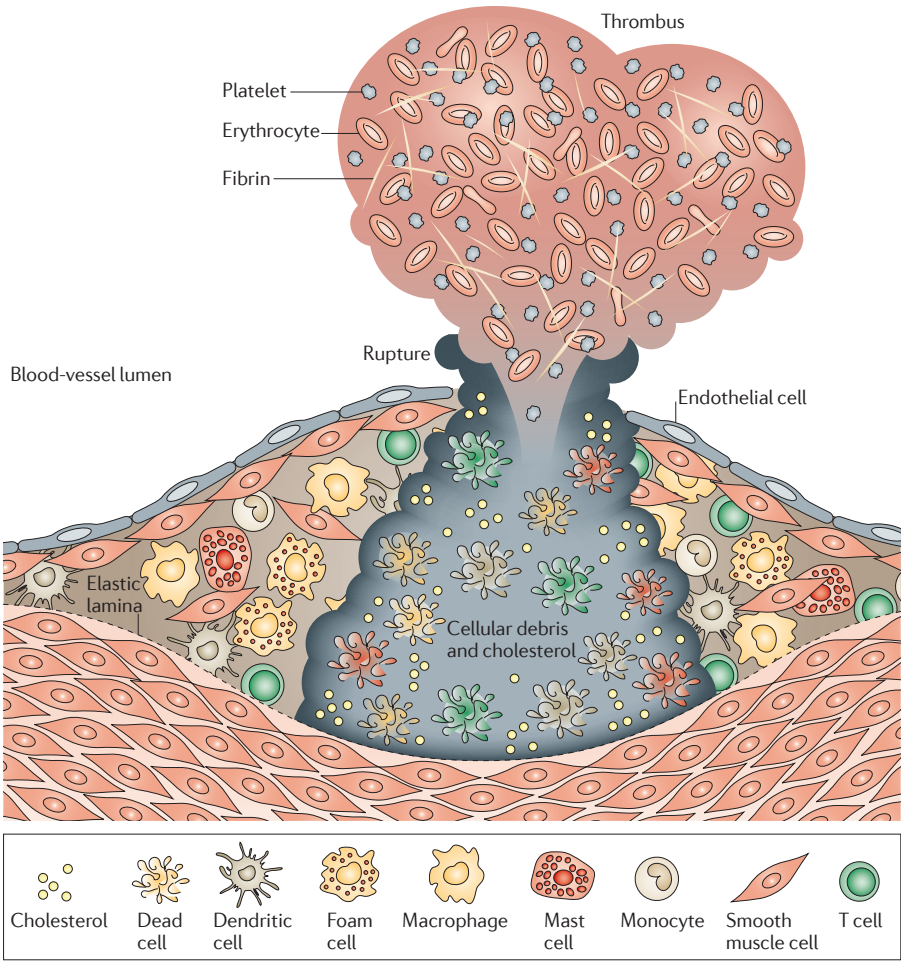

Figure 2 | **Plaque activation, rupture and thrombosis.** When activated, immune cells including macrophages, T cells and mast cells can release pro-inflammatory cytokines, which reduce collagen formation and induce the expression of tissue factor. Proteases that attack the collagenous cap are also released by activated immune cells. The weakened plaque might fissure when subjected to the forces of arterial blood pressure. Exposure of subendothelial structures and procoagulants such as tissue factor promotes platelet aggregation and thrombosis. A thrombus forms and might occlude the lumen of the artery, leading to acute ischaemia. Image is reproduced, with permission, from REF. 43 © (2006) Macmillan Publishers Ltd. All rights reserved.

Although the influence of T cells on immunity is undoubted, macrophage heterogeneity is still apparent in T cell-deficient hosts, and antigen-presenting cells may themselves determine T cell heterogeneity. Above all, our knowledge of human tissue macrophage heterogeneity is surprisingly limited, even in the case of major haematopoietic organs (such as the spleen), where the heterogeneity is different to that in rodent models. As information from systems biology floods in, we will require new technologies to dissect the functional relevance of macrophage heterogeneity *in vivo* by non-invasive and invasive methods. The identification of new pathways, mechanisms and targets will then make functionally heterogeneous macrophage subpopulations amenable to selective therapeutic manipulation.

2006 The immune response in atherosclerosis: a double-edged sword, revisited
Peter Libby and Göran K. Hansson. Our 2006 Review highlighted the yin and yang of innate and adaptive immunity as modulators of atherosclerosis[43]. We highlighted the evidence supporting a pro-atherogenic role for T_H1 cells and their signature cytokines, versus a possible mitigating role for a T_H2-polarized immune response and for T_{Reg} cells (and, hence, transforming growth factor-β (TGFβ)), and a putative protective effect of humoral immunity. This postulated tug-of-war between different arms of the innate and adaptive immune systems generally has withstood the test of time (FIG. 2). However, new data have challenged some previously prevalent notions of the stimuli for the adaptive immune response during atherogenesis.

In particular, recent findings suggest that native low-density lipoprotein (LDL), rather than oxidized LDL, is a pathogenic antigen in the context of atherosclerosis[44]. These results suggest that a break in tolerance, rather than the generation of a new antigen, leads to the activation of T_H1 cells, at least in atherosclerotic mice. Furthermore, a restricted set of variable regions in the mouse T cell receptor appears to govern the immune response to native LDL[44]. These new findings, while reinforcing that adaptive immunity can promote atherogenesis, have challenged earlier simplistic suppositions regarding the nature of the responsible antigens. Other recent findings have expanded the cast of combatants in the immune battle raging in the atherosclerotic plaque to include T_H17 cells and IL-17. However, whether T_H17 cells augment or ameliorate atherogenesis remains controversial[45].

Since 2006, biomarkers of inflammation have become more broadly accepted in gauging cardiovascular risk[46]. Moreover, one particular biomarker of innate immune activation — C-reactive protein — has proven useful in targeting the use of preventive therapies that can improve outcomes, a gratifying clinical application of basic science advances in understanding inflammation and immunity in atherosclerosis[47].

With respect to the therapeutic implications of the immune responses that occur in atherosclerosis, our 2006 article pointed to a possible direct anti-inflammatory effect of statins as a contributor to their consistent clinical benefit in patients at risk for atherosclerotic cardiovascular complications. In the intervening years, the evidence for such pleiotropic effects of statins has strengthened[48]. Nonetheless, even when receiving high-dose statins and other current standard therapies, many individuals still have an unacceptably high susceptibility to atherosclerotic events. Direct immunomodulatory or anti-inflammatory interventions have now entered clinical evaluation as novel therapeutic approaches to this residual burden of risk. Trials of passive immunization by transfer of specific antibodies to atherosclerosis-related antigens have entered early clinical evaluation, and active immunization strategies are also being developed. Moreover, both a broadly anti-inflammatory intervention (weekly low-dose methotrexate) and a targeted anti-cytokine strategy (neutralization of IL-1β) will soon undergo rigorous evaluation as anti-atherosclerotic approaches in large-scale clinical trials[49].

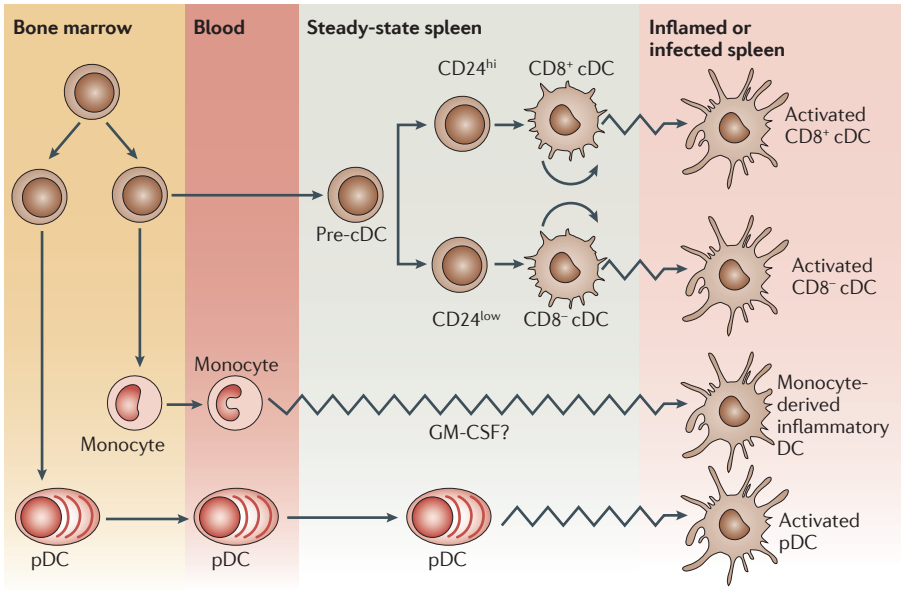

Figure 3 | **Pathways to splenic dendritic cells.** Many, branching pathways are involved in generating the dendritic cells (DCs) found in the spleen. The conventional DCs (cDCs) in the spleen of steady-state mice derive from an intrasplenic precursor, a pre-cDC. This precursor population might be replenished from earlier precursor cells that are generated in the bone marrow, which might occasionally seed the spleen from the bloodstream. Alternatively, as the spleen remains a haematopoietic organ in mice, the pre-cDCs might be generated endogenously. A late branch in the cDC developmental pathway, detected by high or low expression of CD24 on the precursor cells, leads to pre-cDCs in the spleen that are pre-committed to form either $CD8^+$ or $CD8^-$ cDCs, respectively. The cDCs so formed are in an immature state and are still capable of some homeostatic proliferation. In contrast to the cDCs, the plasmacytoid DCs (pDCs) are generated in the bone marrow by a pathway that branches off from that of cDCs. The pDCs found in the mouse spleen and other tissues probably arrive there from the bloodstream. This steady-state situation changes after microbial stimulation or inflammation. In addition to full activation of the resident cDCs and the pDCs in the spleen, a new type of 'inflammatory DC' is then generated from monocytes, a DC type that is not present in the steady state. GM-CSF, granulocyte/macrophage colony-stimulating factor. Image is reproduced, with permission, from REF. 50

Continued work has thus reinforced the importance of inflammation and immunity in atherosclerosis, but as is often the case in scientific endeavours, the data have led us down a path of unanticipated twists and turns. The tantalizing goal of reaping the medical benefits of progress in the fundamental understanding of the double-edged sword of the immune response in atherosclerosis has come a bit closer to reality.

2007 Dendritic cell development — 4 years on

Ken Shortman. The interface between different disciplines is often a rewarding zone for research. Shalin Naik and I certainly found this to be the case while working on our Review of steady-state and inflammatory DC development[50], a research area on the boundary between haematology and immunology. DC development was and remains a complex subject, owing to the multiple specialized subsets of DCs and their developmental plasticity (FIG. 3). Our attempt to bring some logic and order to the information then available must have been appreciated, given the impact of this Review.

There has of course been substantial progress in the field since our Review in 2007. One positive development is the identification of a human subset equivalent to mouse $CD8^+$ DCs[51], which gives hope that the developmental pathways of the mouse and human DC systems will prove to be similar. In our Review, we had avoided considering the DCs of the mucosal immune system, because we found the available data confusing. However, the past 4 years have seen a great advancement in our understanding of the similarities between the migratory DCs found in skin, lung and gut tissues[52].

Although many of the concepts presented in the Review remain valid, in some cases the picture has changed. One generalization we made was that many tissues generate their DCs locally from a reservoir of

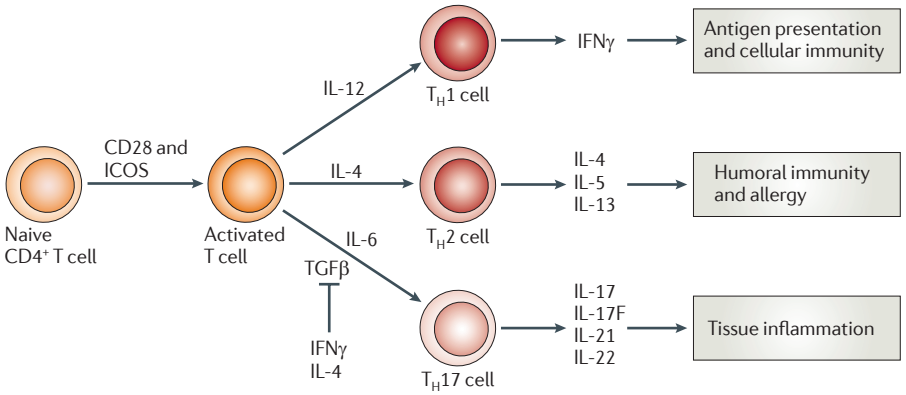

Figure 4 | **General scheme of T-helper-cell differentiation.** Naive CD4+ T cells, after activation by signalling through the T-cell receptor and co-stimulatory molecules such as CD28 and inducible T-cell co-stimulator (ICOS), can differentiate into one of three lineages of effector T helper (T_H) cells — T_H1, T_H2 or T_H17 cells. These cells produce different cytokines and have distinct immunoregulatory functions. Interferon-γ (IFNγ) produced by T_H1 cells is important in the regulation of antigen presentation and cellular immunity. The T_H2-cell cytokines interleukin-4 (IL-4), IL-5 and IL-13 regulate B-cell responses and anti-parasite immunity and are crucial mediators of allergic diseases. T_H17 cells have been shown to express IL-17, IL-17F, IL-21 and IL-22 (and IL-26 in humans) and to regulate inflammatory responses. TGFβ, transforming growth factor-β. Image is reproduced, with permission, from REF. 57 © (2008) Macmillan Publishers Ltd. All rights reserved.

immediate precursors, rather than depending on a continuous input of precursors from the bone marrow. This remains true for the skin DCs known as Langerhans cells. However, the apparent (and we wrote "surprising") independence of spleen DCs from bone marrow input has been shown to be incorrect[53]. Although there is some limited local DC proliferation and maturation in the spleen, maintenance of the spleen DC population does require a continuous input of pre-DCs from the bone marrow.

Our knowledge of the developmental pathway that gives rise to steady-state DCs in the lymphoid tissues and plasmacytoid DCs has recently taken a step forward, with the isolation of a common DC precursor (CDP)[54] (also termed pro-DC[55]) that exists upstream of the pre-DC. In current consensus models, the precursor further upstream of the CDP is the macrophage–DC precursor (MDP), which was already considered in our Review. The MDP is a neat way of linking the DC and macrophage developmental pathways. However, no clonal data are available to demonstrate that a single restricted precursor of this isolated population gives rise to both macrophages and DCs (steady-state lymphoid tissue-resident DCs and plasmacytoid DCs). We currently dissent from the concept of a common restricted MDP and believe that these cell types develop independently from a myeloid precursor. A clearer picture of the developmental pathways that operate *in vivo* should soon be obtained by my colleague Shalin Naik, who is applying the molecular 'barcoding' techniques of Ton Schumacher[56] to track DC development.

Mapping DC development at the cellular level by identifying intermediate precursors provides useful signposts along the developmental pathway. However, a full understanding of DC development must now proceed beyond the cellular to the molecular level, with an account of the exogenous factors (such as cytokines) and endogenous factors (such as transcription factors) that govern the differentiation processes involved.

2008 Towards understanding T_H17 cell lineage specification

Chen Dong. Effector CD4+ T cells, which were historically divided between T_H1 and T_H2 subsets, welcomed a new brother, T_H17 cells, 5 years ago (FIG. 4). The progress in our understanding of the unique extracellular and intracellular regulators of T_H17 cells that occurred at an unprecedented rate was summarized in my 2008 Review, 'T_H17 cells in development: an updated view of their molecular identity and genetic programming'[57]. At a time when T_H17 cell biology had come to some maturity, I attempted to outline the framework of our then knowledge on T_H17 cell development. The Review was well received, to my great satisfaction, and this was due, at least in part, to the interest in T_H17 cells and their relevance to human diseases beyond the immunology community.

Since then, there has been further development in the field and, in my view, several front lines are worth mentioning here.

The first is the identification of new regulators. IL-1 has been found to directly regulate early T_H17 cell differentiation[58]. In addition, several new transcription factors have emerged as regulators in T_H17 cells, including IκBζ (NF-κB inhibitor-ζ), BATF (basic leucine zipper transcriptional factor ATF-like) and SMAD2 (REF. 59). The importance of commensal bacteria in T_H17 cell generation in the gut has become apparent[60], as has their role in initiating T_H17 cell-mediated autoimmunity[61].

Second, the 'type-17 cytokines' IL-17A, IL-17F and IL-22 have been found to be expressed (although maybe not always simultaneously) by other types of T cell, including T_H22 cells, γδ T cells and natural killer T (NKT cells), as well as by innate lymphocytes, such as NK cells and lymphoid tissue inducer (LTi) cells[62]. Retinoic acid-receptor-related orphan receptor-γ (RORγ) appears to be important for cytokine expression by many, if not all, of these cells. The function and regulation of various populations of type-17 cytokine producers in diverse immune responses is predicted to be an active area of research in the future.

Since the discovery of T_H17 cells, additional T cell subsets, including T follicular helper (T_{FH}) and IL-9-secreting T_H9 cells have arisen. Moreover, the conversion of T_H17 cells to other T_H cell types, especially T_H1 cells, has been witnessed *in vitro* and under certain circumstances *in vivo*[59]. It has also become appreciated that T_{Reg} cells may utilize some T_H17 cell-specific molecular pathways to suppress T_H17 cell-mediated immunopathology[63]. The plasticity of T_H cell subsets and their genetic and epigenetic cross-regulation is predicted to continue as an important area of T cell biology, but it requires new tools for the genetic reporting of distinct subsets and for 'fate mapping'.

Finally, there are ongoing developments in therapeutic targeting. An IL-12/IL-23p40-specific antibody has been approved for the treatment of psoriasis, and the use of an IL-17-specific antibody has shown promising effects in this and other diseases[64]. Three selective inhibitors of RORγ have been reported[65–67], which may be applied to globally inhibit the function of type-17 cytokine producers. New uses for these therapeutic approaches may be explored in the future for other diseases, such as inflammation-associated cancers, in addition to auto-inflammatory and autoimmune disorders. This exciting progress in the clinic justifies all the basic research we have been conducting.

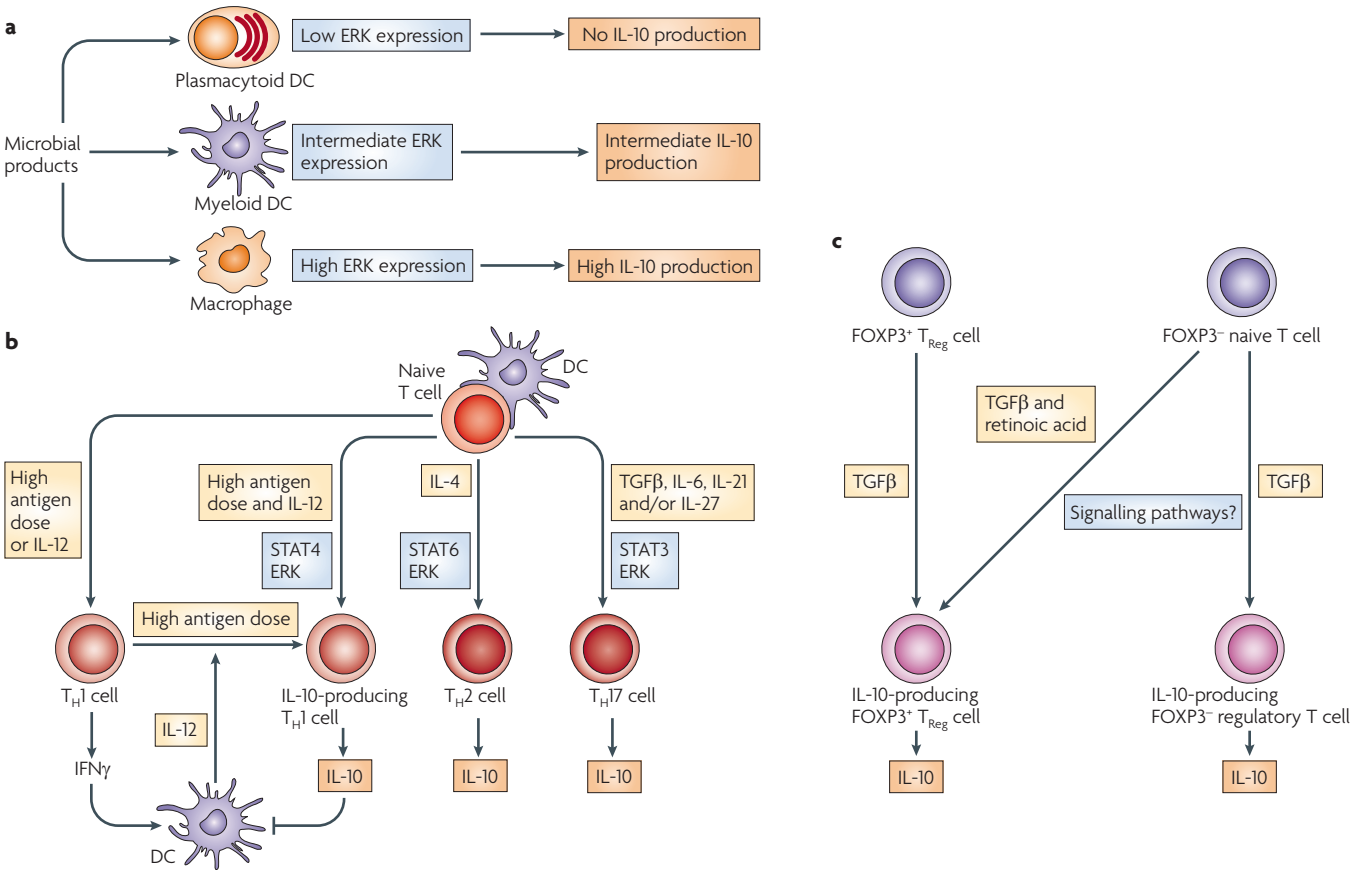

Figure 5 | **Signals that induce interleukin-10 expression by cells of the innate immune response. a** | The expression of interleukin-10 (IL-10) can be induced by Toll-like receptor (TLR) or non-TLR signalling in macrophages and myeloid dendritic cells (DCs). Activation of TLRs and their adaptor molecules — myeloid differentiation primary-response protein 88 (MYD88) and TIR-domain-containing adaptor protein inducing IFNβ (TRIF) — results in the activation of the extracellular signal-regulated kinase 1 (ERK1) and ERK2 (which are collectively referred to here as ERK), p38 and nuclear factor-κB (NF-κB) pathways. Activation of these pathways results in the induction of IL-10 expression, in addition to pro-inflammatory cytokines. In myeloid DCs, non-TLR signals through DC-specific ICAM3-grabbing non-integrin (DC-SIGN) and RAF1 can augment TLR2-induced IL-10 production. Furthermore, activation of dectin 1 and the signalling molecules spleen tyrosine kinase (SYK) and ERK results in IL-10 production. In macrophages, a role for nucleotide-binding oligomerization domain 2 (NOD2) signalling in IL-10 induction, in crosstalk with TLR2, has been described. **b** | Positive and negative feedback loops for IL-10 regulation in macrophages. The p38 and ERK pathways leading to IL-10 expression by macrophages are tightly controlled by interferon-γ (IFNγ) and IL-10 itself. IL-10 feeds back to induce the expression of dual-specificity protein phosphatase 1 (DUSP1), which negatively regulates p38 phosphorylation and thus limits IL-10 production. IL-10 can also positively feed back to upregulate tumour progression locus 2 (TPL2) expression, thus providing a positive amplification loop for its own production. In addition, IFNγ can also interfere with the phosphoinositide 3-kinase (PI3K)–AKT pathway, releasing glycogen synthase kinase 3 (GSK3). As GSK3 normally blocks IL-10 expression by acting on the transcription factors cAMP response element-binding protein (CREB) and activator protein 1 (AP1), IL-10 production is inhibited by IFNγ through its effects on PI3K. IL-10R, IL-10 receptor; MEK, MAPK/ERK1 kinase; MSK, mitogen- and stress-activated protein kinase; RIP2, receptor-interacting protein 2; STAT3, signal transducer and activator of transcription 3; TRAF3, TNFR-associated factor 3. Image is reproduced, with permission, from REF. 79 © (2010) Macmillan Publishers Ltd. All rights reserved.

2009 Continued interest in MDSCs

Dmitry Gabrilovich. The ability of some myeloid cells to suppress immune responses has been known for a long time. However, until recently, the biological significance of these cells was not fully appreciated. This situation has changed during the last 8–10 years, with the discovery of the crucial role of myeloid-derived suppressor cells (MDSCs) in controlling immune responses.

In our 2009 Review[68], we discussed the current knowledge of the nature and biological role of MDSCs. We summarized the evidence indicating that MDSCs are an intrinsic part of the differentiation pathway of myeloid cells and represent a mixed group of cells at different stages of myeloid cell differentiation. These populations are expanded and activated in response to various factors produced in pathological conditions such as tumours, chronic infections, trauma and/or inflammation. This abnormal activation of MDSCs results in their ability to suppress immune responses in antigen-specific and -nonspecific manners. We proposed that MDSCs represent an important general mechanism for regulating immune responses under different pathological conditions. This mechanism is very rapidly initiated during these conditions and prevents uncontrolled immune reactivity. In tumour-bearing hosts, this mechanism is hijacked by the tumour and is used to inhibit antitumour immune responses. Our Review was highly cited, probably because it coincided with a growing interest in these cells among researchers from different fields and because it presented a cohesive view on this novel group of cells and their potential biological functions.

Since the publication of the Review, the interest in the field of MDSCs has continued to grow, with more than 250 new papers published. We have learned new mechanisms by which MDSCs suppress T cells[69] and NK cells, and promote tumour

metastases[70]. New molecular mechanisms that regulate MDSC proliferation have been described in cancer[71,72] and infections[73]. The liver was found to be a reservoir of MDSCs in tumour-bearing mice[74]. Granulocytic MDSCs and tumour-associated neutrophils have been better characterized in cancer[75,76]. The functional compartmentalization of MDSCs in peripheral lymphoid organs and the tumour microenvironment has been demonstrated, as well as their ability to differentiate into tumour-associated macrophages[77]. It was demonstrated that human MDSCs could be generated *in vitro*[71] and, importantly, MDSCs have been shown for the first time to be useful in the context of solid organ transplantation in mice[78].

However, many crucial questions still remain unanswered. Probably the most important address the following key issues: better phenotypic characterization of these cells, especially in humans; better delineation of the nature of these cells vis-à-vis monocytes and neutrophils; identification of specific therapeutic methods to eliminate these cells in cancer; the development of MDSC-based therapy for autoimmune diseases; and the use of these cells in solid organ and stem cell transplantations. We have every reason to believe that the next 10 years will bring new and exciting discoveries associated with these cells.

2010 The regulation of IL-10 production in immune cells

Leona Gabryšová, Ashleigh Howes and Anne O'Garra. IL-10 is an important anti-inflammatory cytokine that prevents inflammatory and autoimmune pathologies by limiting the immune response to pathogens and microbial flora, thereby preventing damage to the host. Many cells of the innate and adaptive immune system produce IL-10; however, the factors that regulate its production in the various cell types are currently unclear. Further complicating the study of IL-10 regulation is the fact that it is induced under feed-forward conditions alongside other cytokines that can modulate its production, giving rise to intricate regulatory networks. This complexity of IL-10 regulation was addressed in our 2010 Review[79].

IL-10 production in innate immune cells (such as macrophages and DCs) and in T cells appears to be regulated by both distinct and common pathways (FIG. 5). Downstream of TLR activation in macrophages and DCs, the tumour progression locus 2 (TPL2; also known as MAP3K8)–extracellular signal-regulated kinase (ERK) pathway is central to the production of IL-10 (REF. 80). This is illustrated by the correlation between the levels of ERK activation and IL-10 production by the different innate cell types (reviewed in REF. 79). IL-10 production downstream of the pattern recognition receptor dectin 1 (also known as CLEC7A) also requires the ERK pathway[81]. In addition, IL-10 is regulated by other signalling pathways, including the p38 and nuclear factor-κB (NF-κB) pathways (reviewed in REF. 79).

Similarly to in macrophages and DCs, ERK signalling is required for IL-10 production by different T_H cell subsets — T_H1, T_H2 and T_H17 cells — indicating that ERK is a common regulator of IL-10 in various immune cell types[82]. However, T_H cell subset-specific transcription factors have also been implicated in IL-10 regulation. The transcription factor GATA-binding protein 3 (GATA3) — which is essential for the differentiation of T_H2 cells but is not expressed in other T_H subsets or in macrophages or DCs — has been shown to remodel the *Il10* gene locus[83]. Other transcription factors shown to regulate IL-10 induction — such as signal transducer and activator of transcription 4 (STAT4) in T_H1 cells[82] and MAF[84,85] — also have the added complication of being involved in T_H cell differentiation. The future challenge, therefore, is to decipher the complex IL-10 regulatory networks in different cell types in response to various stimuli, including the downstream signalling pathways and the transcription factor hierarchies that are specifically involved in *Il10* gene regulation. Furthermore, the role of epigenetics and post-transcriptional regulatory events (such as mRNA stability) in the regulation of IL-10 are only just beginning to be understood (reviewed in REF. 79).

Also of importance is the further characterization of the cellular sources of IL-10 and the timing of its production required to modulate an immune response. The cellular source of IL-10 may indeed vary according to the microorganism or the host. For instance, $CD4^+CD25^+$ T_{Reg} cells have been shown to suppress effector T cell responses by both IL-10-dependent and -independent mechanisms in response to infection with the clinically curable Friedlin strain of *Leishmania major*[86]. By contrast, $CD4^+CD25^-FOXP3^-$ T_H1 cells have been shown to be the major source of IL-10 during infection with the non-healing NIH/Sd clinical isolate of *L. major* from patients with visceral leishmaniasis[87]. Similarly, $T\text{-}bet^+FOXP3^-$ T_H1 cells are the major source of IL-10 during *Toxoplasma gondii* infection[88]. T_H1 and T_H17 cell-mediated immune responses to *Mycobacterium tuberculosis* infection are also hampered by IL-10, although its exact source in this infection is currently unknown[89]. In the gastrointestinal tract, $FOXP3^+$ and $FOXP3^-$ IL-10-producing $CD4^+$ T cells have been reported[90], both of which were recently shown to directly inhibit T_H17 (both $IL\text{-}17^+IFN\gamma^-$ and $IL\text{-}17^+IFN\gamma^+$) cell-mediated inflammatory bowel disease in an IL-10-dependent manner[91,92].

Thus, owing to this immense complexity, the use of traditional biochemical methods together with genome-wide, high-throughput approaches and bioinformatics in *in vitro* and *in vivo* systems is the key to gaining a better understanding of IL-10 regulation in different immune cells. This will then facilitate the fine-tuning of immune intervention strategies.

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Competing interests statement
The authors declare competing financial interests: see Web version for details.

FURTHER INFORMATION

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OPINION

The parallel lives of angiogenesis and immunosuppression: cancer and other tales

Gregory T. Motz and George Coukos

Abstract | Emerging evidence indicates that angiogenesis and immunosuppression frequently occur simultaneously in response to diverse stimuli. Here, we describe a fundamental biological programme that involves the activation of both angiogenesis and immunosuppressive responses, often through the same cell types or soluble factors. We suggest that the initiation of these responses is part of a physiological and homeostatic tissue repair programme, which can be co-opted in pathological states, notably by tumours. This view can help to devise new cancer therapies and may have implications for aseptic tissue injury, pathogen-mediated tissue destruction, chronic inflammation and even reproduction.

The vascular system develops through the coordinated actions of both vasculogenesis and angiogenesis. Vasculogenesis gives rise to *de novo* blood vessels, whereas angiogenesis is the sprouting of new vessels from pre-existing ones. Physiological angiogenesis — which occurs during development and wound healing — proceeds through vessel destabilization, endothelial cell migration and proliferation, and sprouting. This is followed by the resolution phase, which is characterized by reduced endothelial cell proliferation and stabilization of the new vessel. Pathological angiogenesis shares many of the same processes, but is characterized by a failure of the resolution phase and the generation of a highly disorganized vascular network. Pathological angiogenesis is a key feature of tumour biology, but is also involved in a broad spectrum of inflammatory diseases, such as rheumatoid arthritis and connective tissue disorders[1]. Although pathological angiogenesis is generally viewed as a process driven by resident endothelial cells and mobilized endothelial progenitor cells, a complex tissue repair programme is responsible for regulating the process of remodelling and vessel formation. It is our view that pathological angiogenesis is integrated with and co-regulated by immunosuppressive processes in a homeostatic tissue repair programme.

There are numerous examples that demonstrate the existence of a biological response characterized by the simultaneous activation of angiogenesis and immunosuppression. This response can be initiated by diverse physiological stimuli, such as those that occur during aseptic tissue injury resulting from ischaemia–reperfusion injury or wounding, during infection and even during pregnancy. We think that the benefit of such an interconnected and reciprocal tissue repair programme is to ensure tissue homeostasis. Summoning cells that can simultaneously mediate angiogenesis and immunosuppression provides an efficient process that economizes resources at times of homeostatic crisis. This hypothesis is supported by the ever-growing list of haematopoietic cell types that, when appropriately polarized, can promote both immunosuppression and angiogenesis. For example, myeloid-derived suppressor cells (MDSCs)[2], dendritic cell (DC) subsets[3,4], natural killer (NK) cells[5], neutrophils[6], macrophages, B cells[7,8] and regulatory T (T_{Reg}) cells[9], as well as the angiogenic endothelium itself[10], have been shown to have this dual capacity. Furthermore, mediators secreted by these cells — such as vascular endothelial growth factor A (VEGFA) and prostaglandin E2 (PGE2) — have well-known functions in both angiogenesis and immunosuppression.

Tumour development, much like tissue repair and wound healing, requires the development of neovasculature and the suppression of excessive inflammation. It is possible that tumour development proceeds by the co-option of the homeostatic tissue repair programme, promoting concurrent angiogenesis and immunosuppression, and that this becomes the overarching biological programme that drives the polarization of the