The therapeutic potential of positive and negative immune cell co-stimulation during inflammation

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Abstract
Inflammatory cascades are initiated in response to alarm signals that may result from infection, malignant transformation or trauma. Immunity, however, must be controlled; otherwise damage may occur to otherwise healthy tissue within the same microenvironment. Similarly, peripheral tolerance mechanisms must ensure that autoreactive thymic or bone marrow emigrants do not respond upon encounter with the autoantigen. Organized lymphoid structures such as lymph nodes, spleen and Peyer’s patches appear to regulate inflammation successfully, displaying controlled expansion and contraction. However, when immune cells flood into effector sites, the organization of T- and B-lymphocytes is lacking. What controls inflammatory cascades in lymph nodes but rarely in effector sites is not clear. We believe the difference lies in the Toll-like receptor ligand load, which is high in effector sites and drives uncontrolled inflammation. Similarly, we believe that initiation of autoimmune inflammation is initiated by the liberation of inflammatory signals due to infection or trauma. In this review, we highlight some of the molecules responsible for maintaining an activated T-cell phenotype, strategies to interrupt these therapeutically and the impact of ligating inhibitory receptors on antigen-presenting cells.

Introduction
The immune system has evolved to recognize and eradicate pathogens and altered or dying host cells. However, excessive or unregulated immunity is responsible for a wide range of diseases, including transplant rejection, autoimmunity and immune pathology during infection. The latter usually occurs upon first encounter with the organism. Once immunological memory is established, a further infection with the same pathogen usually results in milder disease due to rapid clearance before collateral damage can occur. This is particularly evident during infection with influenza virus. Acute respiratory infections, especially influenza virus infections, are a leading cause of death worldwide. For example, the 1918 influenza pandemic killed more people than the First World War. Most experts now consider the emergence of another pandemic to be inevitable. The outcome of infection depends on whether cross-protective pre-existing immunity is present. In a host without prior immunity, influenza virus replicates unchecked in the respiratory tract, eliciting extensive bystander tissue damage and immune pathology that may ultimately result in death, as shown in Figures 1(A)–1(C). However, if the host has protective immunity, then viral replication is restricted and immune pathology is minimized, as shown in Figures 1(D) and 1(E).

The life-threatening symptoms are caused in part by virus-promoted release of inflammatory cytokines, e.g. IFN-γ (interferon-γ) and TNF (tumour necrosis factor) from NK (natural killer) cells, macrophages and activated T-cells. These cytokines lead to pulmonary oedema and transudation of leucocytes into the alveolar space, which then impair gas exchange, a syndrome termed the ARDS (acute respiratory distress syndrome) [1–3]. Convincing results in mouse models shows that a reduction of pro-inflammatory cytokines (especially TNF) is beneficial, and can greatly increase survival rates [4].

Why such excessive inflammation is allowed to occur is not entirely clear but seems to be a common feature of sites devoid of, or with little, constitutive lymphoid tissue. Organized lymphoid tissue such as lymph nodes, spleen and Peyer’s patches expand greatly during an immune response and yet do not tend to lead to clinical disease. No one ever dies of an inflamed lymph node. Such structures expand and contract in a regulated manner, immune cells are organized and that organization remains regardless of the burden of recruited cells. In addition, immune cells within these organized structures do not tend to produce high levels of inflammatory cytokines. In effector sites lacking organized lymphoid tissue, however, cells flood in during the disease process but are resident in a non-organized manner and produce high levels of inflammatory cytokines. It is not yet clear whether organization equates to control of immunity or whether the diseased or infected effector site promotes un-regulated inflammation. It is intriguing that sites associated with ‘disease’ tend to have the highest TLR (Toll-like receptor) ligand load. During influenza infection for example, the virus replicates...
The outcome of influenza virus infection depends on whether pre-existing cross-reactive immunity exists. In a naïve host, viral infection induces a highly inflammatory environment and APCs loaded with viral antigen track to the draining mediastinal lymph node (MLN) where they activate CD4+ (green) and CD8+ (yellow) T-cells (A). Further inflammatory cytokines, produced due to unchecked viral replication, recruit a large inflammatory infiltrate (B). Memory B- and T-cells (red) then migrate to the spleen, but some will remain resident in the lung (C). In hosts with cross-reactive T- and B-cells, however, antigen-specific cell migration and activation occur rapidly (D). Viral replication is therefore controlled much earlier, reducing bystander tissue damage and cellular infiltration (E).

What therefore prevents ‘inflammation’ in lymph nodes that is lacking in effector sites? Immunity can be loosely categorized into three distinct phases: initiation, maintenance and resolution. Inflammatory disease can occur at each or all of these stages. Excessive initiation due to high antigen load, extensive collateral damage to the affected site or prolonged presence of the antigen will initiate an excessive inflammatory cascade. Similarly, excessive maintenance or retention of the inflammatory infiltrate will cause accumulation of effector cells and lead to disease. Inflammatory disease can equally occur if inflammation does not resolve in a timely manner. In this review, we will discuss some of the regulatory mechanisms that contribute to maintenance or cessation of inflammatory responses, focusing on our expertise in late co-stimulatory signals delivered to antigen-activated T-cells (shown in Figure 2).

The role of OX40 in human and mouse disease and its potential as a therapeutic

OX40 (CD134) is up-regulated on T-lymphocytes 24–48 h after TCR (T-cell receptor) stimulation, and remains expressed for 96 h [5] (for a summary of expression patterns, see Table 1). OX40 is expressed on approx. 10% of T-cells in the peripheral blood of healthy patients [6] and a similar proportion of unstimulated murine splenocytes (E. Gwyer and T. Hussell, unpublished work). During inflammation, the proportion and intensity of OX40 expression increases on T-cells, predominantly at the actual site of inflammation. For example, during lung influenza infection, OX40 expression is highest on T-cells infiltrating the airways where the viral antigen load and inflammatory cytokine production are the highest [7]. Very little expression is observed in the draining lymph node, nor is it up-regulated on T-cells in the blood. Either up-regulation of late co-stimulatory molecules represents a final checkpoint to confirm that the cells are fully activated effector cells before they are allowed into the inflamed organ or the inflammatory microenvironment itself induces their expression. OX40 expression at the affected site is also observed during autoimmune inflammation [6]. For example, immunohistochemical staining for OX40 in mice suffering from EAE (experimental autoimmune encephalomyelitis) shows substantial expression in the inflammatory foci of the white matter in the spinal cord [8]. This up-regulation is again specific to the site of inflammation [9] and only occurs on myelin-specific T-cells [10].

In the few human diseases examined, the level and kinetics of OX40 expression appear to be similar to their
Figure 2 | Reciprocal regulation between T-cell and APCs
T-cells activated via their TCR by MHC/peptide antigen complexes (MHC) and the co-stimulatory signal between CD28 on the T-cell and B7 molecules on the APC require subsequent signals to avoid activation-induced cell death. ICOS and OX40 are both induced on antigen-activated T-cells and when ligated by their cognate receptor on the APC transmit a survival signal to the T-cell, resulting in up-regulation of anti-apoptotic genes and the production of further inflammatory cytokines. CD200 on the T-cell, however, binds to CD200R on the APC and transmits a negative signal reducing APC function. OX40L, OX40 ligand.

Table 1 | The distribution of co-stimulatory and inhibitory signals on T-cells and APCs
+; Known expression; −, known absence; ‘?’ unknown. FDC, follicular dendritic cells; OX40L, OX40 ligand.

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The role of ICOS (inducible co-stimulator) in human and mouse disease and its potential as a therapeutic
ICOS is a member of the CD28 superfamily and is transiently expressed following TCR stimulation (see Table 1). It appears on the cell surface earlier than OX40, peaking at 12–24 h after initial activation. For similar reasons to OX40, the transient nature of ICOS expression and its absence on naive and resting memory T-cells makes it a potential target for inhibition during conditions associated with aberrant or excessive T-cell activation.

Blockade of ICOS in vivo ameliorates autoimmunity (EAE, diabetes and collagen-induced arthritis), asthma and allergy and prevents transplant rejection (for a review, see [15]). ICOS−/− mice on a DBA/1 background are resistant to the development of collagen-induced arthritis [16]. In some
cases, ICOS blockade has been performed in conjunction with the disruption of other co-stimulatory molecules. Inhibition of the ICOS–ICOSL (ICOS ligand) interaction alone prolongs the survival of grafts, whereas blockade of ICOS and CD40 simultaneously results in indefinite survival of fully mismatched rat cardiac allografts [17]. The number of occluded vessels, the degree of occlusion of the vessels and lesions in the vascular wall are all reduced. This is likely to reflect a block in the infiltration of ICOS+ cells into the graft and a reduction in the production of pro-inflammatory cytokines.

In murine models of infection, ICOS inhibition reduces Th1 responses to *Listeria monocytogenes* [32], Th2 responses to *Leishmania major* [32], Th1 and Th2 responses to *Leishmania mexicana* [19] and global T-cell responses to *Nippostrongylus brasiliensis* [20]. However, since ICOS appears rapidly after T-cell activation, it is possible that T-cell ablation will be too dramatic and allow the pathogen to escape immune-mediated elimination. We have recently shown that inhibition of ICOS in the murine influenza model leads to uncontrolled viral replication due to ablation of antigen- and non-antigen-specific T-cells (I.R. Humphreys and T. Hussell, unpublished work).

**CD200 in human and mouse disease and its potential as a therapeutic**

Instead of inhibiting activation signals delivered to antigen-activated T-cells, another method of reducing inflammation would be to reduce the activity of APCs (antigen-presenting cells). By reducing APC activity, inflammatory mediators such as reactive oxygen and nitrogen species, chemokines and cytokines would be decreased. We have previously reported that a reduction of inflammatory metabolites from APCs reduces influenza-driven immunopathology [4,21]. Endogenous receptors on APCs exist that transmit inhibitory signals to curtail inflammation. CD200 (previously known as OX2) and its receptor, CD200R, were discovered 20 years ago [22], but it is only recently that their function has been elucidated. CD200 is widely expressed on a range of cell types, including T-cells, B-cells, dendritic cells, macrophages and neurons (for a review, see [23]) and has an intracellular domain so small that it is unlikely that any signal can be transmitted following ligation (see Table 1). Distribution of the receptor is more limited than CD200, restricted to myeloid cells such as macrophages and dendritic cells. Signalling through the receptor imparts a negative signal to the myeloid compartment, reducing the inflammatory immune response. The pivotal role of CD200 in dampening immunity is observed in CD200 knockout mice that have increased numbers of macrophages and suffer from a more rapid onset and severe forms of EAE and collagen-induced arthritis [24].

Harnessing this negative signal through ligation of CD200R–Ig fusion protein prevented arthritis onset and significantly reduced the production of TNF and IFN-γ compared with untreated mice [25]. Therapeutic treatment is also effective, with arrest of disease progress and a reduction in clinical score [26]. The tissue distribution of CD200 and CD200R in mice is similar to that in humans [27], and the inhibitory effects on murine macrophages in vitro are replicated in human cell lines [28]. Although harnessing the inhibitory signal transmitted by CD200R has not yet been translated to human, it remains an attractive possible therapeutic for diseases characterized by an exuberant inflammatory response including autoimmunity, infection and transplant rejection.

**Concluding remarks**

We have highlighted some of the positive and negative influences of ligation receptors on acquired or innate immune cells. T-cell survival can be reduced by inhibiting late co-stimulatory signals such as OX40 or ICOS; the latter, however, may lead to reduced pathogen clearance as it is expressed early during T-cell activation on antigen- and non-antigen-specific cells. 4-1BB is another late co-stimulatory signal expressed with similar kinetics and is likely to represent another important therapeutic target during inflammatory disease [29,30]. Alternatively, exuberant T-cell activation can be dampened indirectly by reducing the activity of APCs (by ligating inhibitory receptors such as CD200R). Although we have focused on reducing immunity, it is equally possible to enhance it by taking the opposite approach. For example, immunity to *Cryptococcus neoformans* or tumour antigens can be enhanced by ligating OX40 on antigen-activated T-cells with agonistic reagents [31,32]. Whether enhancing or reducing immunity, harnessing receptors displayed by T-cells and APCs provides generic protection. This is an attractive possibility considering the role that the immune system plays in sculpting the phenotype of tumour antigens and pathogens.

**References**


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