IN THE NEWS

Gulf-war vaccination

'...classified 'secret' injections [given to soldiers before deployment in the 1991 Gulf War] ... have most probably led to the development of autoimmune-induced osteoporosis.'

This is the claim made in a leaked report for the war pensions agency by Lieutenant Colonel Graham Howe, clinical director of psychiatry with the British forces health service in Germany (*The Times*). This is the first official support for such a link.

The British Ministry of Defence (MoD) has rigorously denied the existence of a specific Gulf war syndrome — '...Gulf veterans do not present with a distinct and identifiable pattern of symptoms or signs.' (MoD website). For example, veterans have complained of a range of symptoms, from respiratory and digestive problems to memory and psychological problems (*Reuters*). Backed up by independent studies — such as a report by the Medical Research Council in May 2003 (*BBC News*) — the MoD also refutes any link between illness and vaccination.

However, Lt Col Howe describes the case of Lance-Corporal Alex Izett, who received vaccines against anthrax, botulism, plague and other unspecified biological agents in anticipation of war, but was not eventually required to fight. As the subject was not involved in fighting, the report states that the common factor linking the osteoporosis with which he suffers to the high incidence of osteoporosis in Gulf-war veterans is the vaccines he received.

Much of the press coverage has focused on the 'cocktail' of vaccines that soldiers are thought to have received, with some reports that 28 'jabs' were given. The coverage has been sufficient for this controversial issue to be raised in the House of Lords by Labour peer Lord Morris of Manchester (*The Guardian*).

Kirsty Minton

AUTOIMMUNITY

BCR and TLR9 cooperation in autoimmunity

The binding of haptenated CpG double-stranded (ds)DNA to hapten-specific B cells induces a suboptimal signal from a minimally crosslinked B-cell receptor (BCR). The BCR also functions to shuttle the CpG DNA antigen to an intracellular compartment containing Toll-like receptor 9 (TLR9). TLR9 signalling through a MyD88-dependent pathway, together with the signal from the BCR, leads to B-cell activation and proliferation.

Systemic lupus erythematosus (SLE) and other autoimmune diseases often involve a response to immune complexes containing DNA or chromatin-associated proteins. A new study published in *Immunity* has now set out to explain how the autoreactive B cells involved are activated and the exact nature of their specificity.

The AM14 autoimmune mouse strain produces B cells that recognize autologous IgG2a specific for DNA. In particular, the IgG2a antibodies that are recognized can complex with chromatin released from dying cells to form multivalent DNA-containing immune complexes (chromatin-ICs). Previous experiments have shown that in addition to B-cell receptor (BCR) recognition of IgG2a, the DNA portion of chromatin-ICs is involved in the activation of AM14 B cells through Toll-like receptor 9 (TLR9). TLR9 recognizes hypomethylated CpG-containing bacterial DNA, but such motifs are rare in mammalian DNA. The authors set out to investigate this system further using a series of defined haptenated double-stranded DNA fragments (such as NIP2-CG50-NIP) recognized by anti-hapten (NIP-specific) antibodies.

First, what are the relative roles of TLR9 and BCR signalling? Anti-NIP–NIP2-CG50-NIP immune

TUMOUR IMMUNOLOGY

STAT3 – a potential target for tumour immunotherapy

Inhibitors of signal transducer and activator of transcription 3 (STAT3) could be used to activate immune responses against tumours, according to a report now published in *Nature Medicine*.

Although tumour growth can activate inflammatory responses, the immune system generally ignores established cancers. As STAT3 is constitutively activated in many cancers, Wang *et al.* decided to investigate whether activity of this transcription factor could be involved in blocking the initiation of anti-tumour immune responses.

First, the authors observed that disruption of STAT3 signalling led to a selective increase in the production of pro-inflammatory mediators, including tumour-necrosis factor, interleukin-6 (IL-6) and the chemokine CCL5 by tumour-cell lines. By contrast, constitutive expression of STAT3 led to the inhibition of cytokine and chemokine production by tumour cells.

Next, they studied the effect of the pro-inflammatory mediators that are produced by STAT3-disrupted tumours on cells of the immune system. Cells of the innate immune

system, including macrophages and neutrophils, were activated to produce further pro-inflammatory mediators after exposure to the supernatant from tumour cells in which STAT3 was blocked. Furthermore, this supernatant also activated dendritic cells (DCs), which were then able to stimulate tumour-antigen-specific naive T cells to proliferate and produce IL-2 and interferon- γ . These results show that blocking STAT3 signalling in tumour cells results in the activation of both innate and adaptive anti-tumour immune responses.

Further experiments looked at the effects of constitutive expression of STAT3 by tumour cells. In addition to inhibiting the production of pro-inflammatory mediators by these cells, the authors showed that constitutive STAT3 activity induced tumour cells to

complexes stimulated AM14 B-cell proliferation, but neither antibody nor DNA fragment alone was able to do so. Blocking signalling through TLR9 inhibited this response. However, stimulation with DNA fragments plus an IgG2a antibody that does not form immune complexes with DNA did not lead to B-cell activation, indicating that independent ligation of cellular receptors by monovalent antibody and DNA is not sufficient for activation. The authors speculated that BCR-mediated uptake of DNA facilitates stimulation of intracellular TLR9, but it was unclear whether the BCR was acting as a passive shuttle or whether BCR crosslinking had a role in B-cell activation. Using a monosubstituted DNA fragment (NIP-CG50) that is unable to form multivalent immune complexes, it was shown that BCR crosslinking is not essential for B-cell activation in this system. However, signals from the BCR do contribute to stimulation by chromatin-ICs from dying cells, which have a lower frequency of CpG motifs than fragment-ICs, as shown by the partial inhibition by cyclosporin A. It seems that signals from both the BCR and TLR9 combine to give an optimal response.

Second, what is the precise ligand that is recognized by TLR9? Immune

complexes formed from three types of haptened DNA fragment that differ in terms of immunostimulatory CpG content were compared, and only the CG50 fragments, which contain the highest density of canonical immunostimulatory CpG motifs, induced a strong B-cell proliferative response. Methylation of the DNA completely blocked its stimulatory capacity. Therefore, the rare hypomethylated DNA motifs found in mammalian DNA, which the authors suggest might be enriched in apoptotic chromatin, are an essential component of these immune complexes.

However, for chromatin-ICs to form *in vivo* and trigger disease, B cells must first produce DNA-specific antibodies. Importantly, the authors show that the proposed model of BCR/TLR9 co-operation can be extended to DNA-specific B cells (see figure). They suggest that this might also be a general mechanism for the recognition of opsonized foreign antigens such as bacterial DNA.

Kirsty Minton

References and links

ORIGINAL RESEARCH PAPER Viglianti, G. A. *et al.* Activation of autoreactive B cells by CpG dsDNA. *Immunity* **19**, 837–847 (2003)

FURTHER READING Shlomchik, M. J. *et al.* From T to B and back again: positive feedback in systemic autoimmune disease. *Nature Rev. Immunol.* **1**, 147–153 (2001)

produce a diverse range of factors, including IL-10 and vascular endothelial growth factor, that inhibited DC maturation. Interestingly, a common point of convergence of the inhibitors of immune activation was the induction of STAT3 in the DCs themselves, indicating a cascade of STAT3 activation first in the tumour and then in the DCs.

This study highlights the role of STAT3 in regulating innate and adaptive immune responses to tumours, and, as the authors conclude, indicates that blocking STAT3 signalling with specific inhibitors could have direct anti-tumour effects.

Jenny Buckland

References and links

ORIGINAL RESEARCH PAPER Wang, T. *et al.* Regulation of the innate and adaptive immune responses by Stat-3 signaling in tumor cells. *Nature Med.* **10**, 48–54 (2004)

IN BRIEF

T-CELL ACTIVATION

Evidence that structural rearrangements and/or flexibility during TCR binding can contribute to T cell activation.

Krogsgaard, M. *et al. Mol. Cell* **12**, 1367–1378 (2003)

Although the ability of a peptide to activate a T cell often correlates with the half-life of the T-cell-receptor (TCR)–peptide–MHC-complex interaction, not all peptides conform to this rule. Krogsgaard *et al.* studied peptide analogues of a moth cytochrome *c* (MCC) peptide, two of which — K2 and K3 — were more stimulatory than predicted. Thermodynamic analysis of the TCR–peptide–MHC interaction indicated greater changes in heat capacity for K2 and K3, compared with MCC. These changes were associated with structural rearrangement and flexibility after TCR binding. By considering both the half-life of TCR binding and the change in heat capacity, the authors could predict the ability of a peptide to activate a T cell, indicating that conformational change and flexibility contribute to T-cell activation.

AUTOIMMUNITY

Autoimmunity is triggered by cPR-3 (105–201), a protein complementary to human autoantigen proteinase-3.

Pendergraft III, W. F. *et al. Nature Med.* **10**, 72–79 (2004)

Antibodies specific for neutrophil granule antigens, including proteinase 3 (PR3), are associated with autoimmune vasculitis. The authors of this paper detected antibodies specific for complementary PR3 (cPR3) — a peptide translated from the anti-sense DNA strand of the *PR3* gene — in the sera of several patients with vasculitis with PR3-specific antibodies, but not in those without PR3-reactive antibodies or healthy individuals. The cPR3- and PR3-specific antibodies did not cross-react with the same antigen but formed an idiotypic pair. Mice immunized with human recombinant cPR3 developed both cPR3- and PR3-specific antibodies, indicating that peptides translated from the anti-sense strand of an autoantigen, or microbial-derived peptides that mimic this complementary peptide, could initiate autoimmunity.

LEUKOCYTE SIGNALLING

Analysis of C5a-mediated chemotaxis by lentiviral delivery of small interfering RNA.

Hwang, J. I. *et al. Proc. Natl Acad. Sci. USA* **101**, 488–493 (2004)

The authors used small interfering RNA to establish the role of specific components of the intracellular heterotrimeric G-protein complex in the signalling pathway that mediates chemotaxis. Knockdown of the Gβ2 subunit in the mouse macrophage-cell line J774A.1 abolished migration towards C3a and C5a, whereas cells lacking other Gβ or Gα subunits migrated normally. Gβ2 knockdown diminished the C5a-induced Ca²⁺ signal and eliminated sustained Akt phosphorylation, providing evidence that phospholipase-Cβ and Akt are downstream effectors of this signalling pathway. However, in the absence of Gβ2, levels of both Gγ2 and Gγ5 were downregulated, indicating that further studies are required to define the precise molecular mechanisms by which Gβ2 regulates C5a-induced chemotaxis.