IN BRIEF

IMMUNOTHERAPY

Optimization of a self antigen for presentation of multiple epitopes in cancer immunity.

Guevara-Patiño, J. A. *et al. J. Clin. Invest.* **116**, 1382–1390 (2006)

Activation of T cells that recognize self-antigens that are expressed by cancer cells is limited by T-cell tolerance to self-antigens. The authors examined the possibility that enhanced antigen presentation of multiple epitopes could generate multivalent $CD8^+$ T-cell responses. To do this, rationally selected point mutations that create altered peptide ligands were introduced into the gene that encodes tyrosinase-related protein 1 (TYRP1), which is a non-immunogenic self-antigen that is highly expressed by melanoma cells. These mutations caused enhanced protein trafficking, and processing and presentation of various epitopes. Immunization of mice with mutant *Tyrp1* DNA after tumour challenge elicited a multi-epitope, $CD8^+$ T-cell response that led to rejection of a melanoma that was only weakly immunogenic and to prolonged survival. Therefore, rationally designed DNA vaccines can elicit T-cell responses against multiple non-mutated epitopes of the self-antigen.

PHAGOCYTOSIS

Apoptotic cells promote macrophage survival by releasing the anti-apoptotic mediator sphingosine-1-phosphate.

Weigert, A. *et al. Blood* 11 May 2006 (doi:10.1182/blood-2006-04-014852)

Phagocytosis of apoptotic cells by macrophages is an integral part of maintaining cellular homeostasis. Once they have engulfed apoptotic cells, macrophages are protected from apoptosis. Weigert *et al.* have attributed this protection to the release of sphingosine-1-phosphate (S1P) by the apoptotic cell. This protection of macrophages by S1P involves the activation of survival signals that depend on phosphatidylinositol 3-kinase (PI3K), extracellular-signal-regulated kinase (ERK) and calcium. Upregulation of the anti-apoptotic proteins B-cell lymphoma 2 (BCL-2) and BCL-X_L, as well as inactivation of the pro-apoptotic protein BCL-2-agonistic of cell death (BAD), are also involved in this protective process. Therefore, apoptotic cells have an active role, through the secretion of S1P, in preventing apoptosis of phagocytes, such as macrophages.

MUCOSAL IMMUNOLOGY

Postnatal acquisition of endotoxin tolerance in intestinal epithelial cells.

Lotz, M. *et al. J. Exp. Med.* **203**, 973–984 (2006)

The functional relevance of Toll-like receptor (TLR) expression by intestinal epithelial cells (IECs) remains unresolved. Lotz *et al.* showed that fetal, neonatal and adult IECs all expressed the TLR4–MD2 receptor complex. However, only fetal IECs responded to lipopolysaccharide (LPS) stimulation. Postnatal acquisition of LPS tolerance was preceded by a rapid, strong but transient activation of IECs by endogenous endotoxin. Importantly, this spontaneous activation was only observed in vaginally born mice and not in mice delivered by Caesarean section. The postnatal loss of LPS responsiveness was associated with a downregulation of the TLR-signalling molecule interleukin-1-receptor-associated kinase 1 (IRAK1). This phenomenon of acquired unresponsiveness is unique to postnatal IECs, as intestinal macrophages show a constitutive, age-independent, unresponsive phenotype.

IMMUNOTHERAPY

Co-operation for tumour targeting

Greater understanding of the molecular basis of apoptosis and T-cell activation has aided the development of therapeutic strategies for cancer treatment. Now, new research has identified a combination of antibodies, targeting both tumour cells and immune cells, that is effective at eradicating established tumours in mice.

An agonistic monoclonal antibody specific for death receptor 5 (DR5), the receptor for the pro-apoptotic ligand TRAIL (tumour-necrosis-factor-related apoptosis-inducing ligand), has previously been identified as a potential tumour-specific therapy. In addition, T-cell-based immunotherapy for cancer that involves the induction of tumour-specific cytotoxic T lymphocytes (CTLs), through the use of agonistic monoclonal antibodies specific for co-stimulatory molecules such as CD40 and CD137 (also known as 4-1BB), has also generated remarkable interest. Therefore, Uno *et al.* combined monoclonal antibodies specific for DR5, CD40 and CD137 (collectively referred to as trimAb) and assessed the therapeutic potential of this combination for the treatment of established tumours in mice.

TrimAb therapy induced the rejection of established subcutaneous 4T1 mammary tumours in a substantially higher percentage of mice than single or combination monoclonal antibody treatments did. In addition, only treatment with the three monoclonal antibodies together resulted in rejection of established primary fibrosarcomas initiated with the carcinogen 3-methylcholanthrene (MCA). Treatment with trimAb was not associated with any apparent toxicity or induction of autoimmunity. When T cells from the draining lymph node of trimAb-treated mice were stimulated with 4T1 tumour cells, a population of tumour-specific $CD8^+$ T cells that produced high levels of interferon-γ was identified, indicating that trimAb therapy enhances tumour-specific induction of effector CTLs.

NATURAL KILLER CELLS

Adaptive killers

Adaptive immunity depends on the presence of B cells and T cells. Or does it? Now von Andrian and colleagues show that in the classic example of adaptive immunity, the hapten-induced contact hypersensitivity (CHS) response, hepatic natural killer (NK) cells can mediate antigen-specific, long-lived adaptive recall responses independently of B cells and T cells.

A widely used model of CHS involves sensitization of mice by painting the hapten 2,4-dinitrofluorobenzene (DNFB) on to the dorsal skin, followed by challenge with DNFB at a remote location (such as the ear) several days later. Challenge after sensitization induces a local hapten-specific recall response that has previously been associated with αβ T cells, γδ T cells and B1 cells. However, O'Leary *et al.* found that the magnitude of the sensitization-dependent recall response was identical in recombination-activating gene 2 knockout (*Rag2*$^{-/-}$) mice, which are completely devoid of B cells and T cells. They also found that the immune response to hapten in these *Rag2*$^{-/-}$ mice was inducible, long-lived and antigen-specific, all of which are functional hallmarks of adaptive immunity.

Several observations indicated that the most probable cell type involved in this B-cell- and T-cell-independent CHS response was NK cells. Therefore, to confirm the role of this cell type, NK cells were depleted from *Rag2*$^{-/-}$ mice using asialo-GM1-specific or NK1.1-specific antibody. These mice, when challenged with DNFB following sensitization, had completely lost the ability to mount a CHS response.

To further confirm these findings, sensitized NK cells were adoptively

Using R331 renal carcinoma cells that express caspase-8 (FLICE)-like inhibitory protein (FLIP), which inhibits TRAIL–DR5-mediated apoptosis, the authors showed that DR5-mediated tumour-cell apoptosis was essential for trimAb-mediated tumour rejection. Interestingly, only a minor population (10%) of DR5-susceptible tumour cells in the tumour burden was necessary for tumour rejection to occur in response to trimAb therapy. This rejection required the activation of $CD8^+$ T cells, indicating that both tumour-cell apoptosis through DR5 and tumour-specific CTL responses were required for tumour eradication by trimAb therapy.

So, combining agonistic monoclonal antibodies that activate T cells (CD40 and CD137) with an agonistic monoclonal antibody that induces tumour-cell apoptosis (DR5) is an effective tumour-specific immunotherapy in mice. This combination therapy might be a potential strategy for cancer immunotherapy in humans.

Olive Leavy

ORIGINAL RESEARCH PAPER Uno, T. *et al.* Eradication of established tumors in mice by a combination antibody-based therapy. *Nature Med.* 7 May 2006 (doi:10.1038/nm1405)

transferred to naive mice that were deficient for both RAG2 and the common cytokine receptor γ-chain. These mice, in addition to being B-cell- and T-cell-deficient, also completely lack NK cells. Subsequent challenge with DNFB resulted in a vigorous CHS response in these mice, confirming the ability of sensitized NK cells to mediate a hapten-specific memory response independently of B cells and T cells. These transferable hapten-specific NK memory cells were shown to localize in the donor's liver but not in the spleen.

These results describe an unexpected function for NK cells and indicate that a B-cell- and T-cell-independent adaptive immune response exists in mammals.

Olive Leavy

ORIGINAL RESEARCH PAPER O'Leary, J. G., Goodarzi, M., Drayton, D. L. & von Andrian, U. H. T cell- and B cell-independent adaptive immunity mediated by natural killer cells. *Nature Immunol.* **7**, 507–516 (2006)

CYTOKINES

IL-25 expels worms

Two papers published recently in *The Journal of Experimental Medicine* provide new insight into the development of protective T helper 2 (T_H2)-cell responses *in vivo*: they show that the cytokine interleukin-25 (IL-25) is an important regulator of T_H2-cell responses.

It has been suggested that IL-25 (also known as IL-17E) is involved in the development of T_H2-cell responses. Because immunity to infection with parasitic helminths is usually associated with T_H2-cell responses, both groups studied the role of IL-25 in immunity and inflammation *in vivo* using mouse models of infection with such parasites.

Owyang *et al.* showed that, although AKR mice are normally susceptible to infection with *Trichuris muris*, treatment with recombinant IL-25 induced resistance to infection with this parasite. Resistance to infection was associated with a T_H2-cell response, including increased production of IL-4, decreased production of interferon-γ (IFNγ) and increased numbers of goblet cells in the caecum. Consistent with these observations, although wild-type C57BL/6 mice infected with *T. muris* mount an efficient T_H2-cell response and are resistant to infection, IL-25-deficient C57BL/6 mice infected with *T. muris* failed to mount a T_H2-cell immune response and were unable to clear the worms from the gastrointestinal tract.

Similarly, Fallon *et al.* found that IL-25-deficient mice were unable to clear the parasitic helminth *Nippostrongylus brasiliensis* as rapidly as wild-type mice. Delayed clearance of the parasite was associated with delayed induction of T_H2-cell responses. For example, whereas in the presence of IL-25 large amounts of the T_H2 cytokines IL-4, IL-5 and IL-13 were detected 5 days after infection, in the absence of IL-25 cytokines were not observed until 10 days after infection.

Owyang *et al.* found that blocking T_H1-cell responses in IL-25-deficient mice infected with *T. muris* reversed the decrease in production of T_H2 cytokines and enabled the mice to clear the infection. Therefore, in addition to a role in the efficient induction of T_H2-cell responses, IL-25 might have a role in the suppression of T_H1-cell responses. Consistent with this anti-inflammatory role, chronically infected IL-25-deficient mice developed severe intestinal inflammation that was associated with high levels of pro-inflammatory cytokines such as IFNγ and IL-17 in the caecum.

Using mice expressing a lacZ reporter construct in the *Il25* locus, Owyang *et al.* were able to show that subsets of $CD4^+$ and $CD8^+$ T cells in the caecal patch (which is a lymphoid follicle closely associated with the mucosal surface) of mice resistant to infection with *T. muris* express IL-25. Furthermore, Fallon *et al.* showed that a population of non-B-cell, non-T-cell $KIT^+Fc\varepsilon RI^-$ cells responded to IL-25 by producing large amounts of IL-4, IL-5 and IL-13. In resistant mice, the number of these cells in the mesenteric lymph nodes increased following infection with *N. brasiliensis*, whereas in IL-25-deficient mice the number of these cells remained static. The correlation between the number of these cells and the ability of the mice to clear *N. brasiliensis* led the authors to suggest that these cells are an initial source of T_H2 cytokines following infection with *N. brasiliensis*.

These two studies clearly identify IL-25 as a regulator of T_H2-cell responses *in vivo* and, as Owyang *et al.* suggest, provide a new potential therapeutic target for the treatment of diseases associated with pathogenic T_H2-cell responses.

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ORIGINAL RESEARCH PAPERS Owyang, A. M. *et al.* Interleukin 25 regulates type 2 cytokine-dependent immunity and limits chronic inflammation in the gastrointestinal tract. *J. Exp. Med.* **203**, 843–849 (2006) | Fallon, P. G. Identification of an interleukin (IL)-25-dependent cell population that provides IL-4, IL-5, and IL-13 at the onset of helminth expulsion. *J. Exp. Med.* **203**, 1105–1116 (2006)