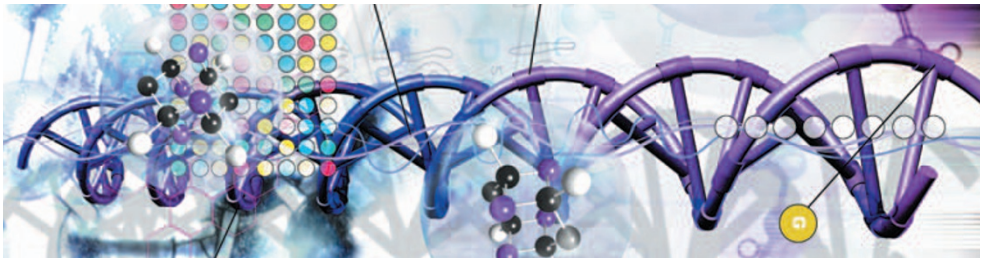


GENE THERAPY

Phoxing a myeloid-cell genetic defect

> this trial resulted in the successful treatment of two individuals with X-linked CGD

Chronic granulomatous disease (CGD) is a primary immunodeficiency disease that is caused by mutation of any of the four genes encoding the subunits of phagocytic oxidase and that is characterized by phagocytes lacking antimicrobial activity. Previous attempts to treat individuals with CGD using gene therapy have been unsuccessful. However, in a new study, published in *Nature Medicine*, successful correction of the gene defect was achieved using non-myeloablative conditioning before infusion of the genetically modified autologous haematopoietic progenitors.

The authors harvested $CD34^+$ progenitors from the blood of two individuals with X-linked CGD (that is, individuals with CGD as a result of mutations in the gene encoding $gp91^{phox}$). These cells were transduced with a γ-retroviral vector expressing the gene encoding $gp91^{phox}$ before being re-infused into the patients, who had been conditioned with a course of non-myeloablative chemotherapy. Gene-modified cells were detected in the peripheral-blood leukocytes of both individuals from day 21 after infusion until the latest time point analysed (542 days after infusion), as well as in the haematopoietic-progenitor population in the bone marrow. Most of the genetically modified peripheral-blood leukocytes were myeloid cells, in particular granulocytes.

Analysis of the sites of retroviral integration indicated that the clonal diversity of the genetically modified cells decreased over time but never became monoclonal. The clones that emerged showed retroviral integration in or near the upstream regulatory regions of at least one of three particular gene loci. Crucially, although these clones expanded preferentially *in vivo*, eventually forming up to 80% of the genetically modified cells, they were unable to proliferate in culture in the absence of growth factors, indicating that they were not able to self-renew.

Expression of $gp91^{phox}$ was detected in granulocytes from the

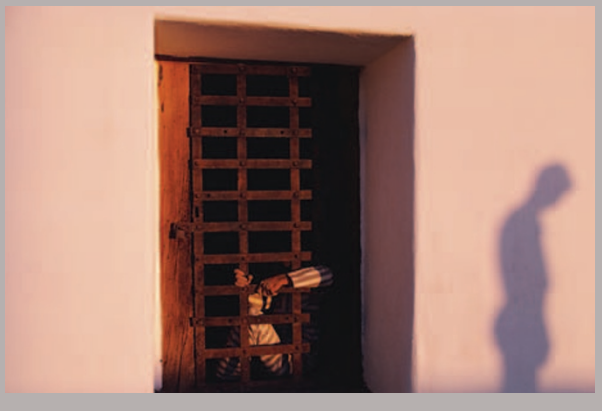
PHAGOCYTOSIS

CRIg clears out the bad guys

Pathogens in the circulation that become coated with fragments of complement component 3 (C3) — a process known as C3 opsonization — are phagocytosed by Kupffer cells (liver-resident macrophages) and are thereby cleared from the host. A report published recently in *Cell* has now identified a new complement receptor, complement receptor of the immunoglobulin superfamily (CRIg; also known as Z39Ig), as the receptor that is required for this efficient phagocytosis of C3-opsonized pathogens by Kupffer cells.

Previous studies indicated that although Kupffer cells express one of the receptors for fragments of C3, complement receptor 3, this receptor does not seem to have a role in the clearance of C3-opsonized pathogens. So, when Helmey *et al.* found that both human CRIg and mouse CRIg were expressed by Kupffer cells and subsets of macrophages resident in other tissues, they set out to determine whether CRIg was the receptor through which Kupffer cells mediate phagocytosis of C3-opsonized pathogens.

There are two alternative splice variants of human CRIg, a long form and a short form, but only a single form of mouse CRIg, which has 67% sequence homology with the short isoform of human CRIg. All three CRIg proteins were shown to bind soluble forms of the C3 fragments C3b and iC3b, as well as particles opsonized with C3b and iC3b. The *in vivo* function of CRIg was analysed using mice lacking expression of CRIg. Although these mice had normal numbers of Kupffer cells and other populations of tissue-resident macrophages, CRIg-deficient Kupffer cells did not bind soluble C3b and iC3b, and, compared with wild-type Kupffer cells, had impaired binding to C3-opsonized IgM-coated sheep erythrocytes. Furthermore, Kupffer cells in CRIg-deficient mice that were infected intravenously with either *Listeria monocytogenes* or *Staphylococcus aureus* internalized fewer bacteria than did Kupffer cells in similarly infected wild-type mice. In addition, more live bacteria were recovered from the blood, spleen and tissues of infected CRIg-deficient mice. This defect in clearance of pathogens from the circulation resulted in increased levels of serum cytokines in CRIg-deficient mice that were infected intravenously with *L. monocytogenes* and in increased mortality.



treated individuals, and NADPH-oxidase activity was reconstituted in these cells. Although the amount of superoxide produced by genetically modified neutrophils from the patients was less than that produced by neutrophils from healthy individuals, it was sufficient to mediate bacterial killing. Furthermore, the two individuals who had received gene therapy showed no evidence of recurrence of the bacterial and fungal infections that they had suffered before treatment.

Although this trial resulted in the successful treatment of two individuals with X-linked CGD, monitoring of the individuals will continue for 3–5 years because of the possibility that the abnormal clonal expansion observed could eventually result in leukaemogenesis.

Karen Honey

ORIGINAL RESEARCH PAPER Ott, M. G. *et al.* Correction of X-linked chronic granulomatous disease by gene therapy, augmented by insertional activation of *MDS1-EVI1*, *PRDM16* or *SETBP1*. *Nature Med.* 2 Apr 2006 (doi:10.1038/nm1393)

Mechanistically, it was shown that CRIg on the surface of Kupffer cells is constitutively being internalized and then recycled back to the cell surface. Consistent with this finding, internalized CRIg was observed to colocalize with transferrin, which is a marker of recycling endosomes, and not with lysosomal-associated membrane protein 1, which is a marker of lysosomes. Further analysis showed that during phagocytosis of C3-opsonized particles, CRIg is recruited to the forming phagosome but returns to the recycling endosomes before, or during, phagosome–lysosome fusion.

This study identifies CRIg as the main receptor expressed by Kupffer cells for pathogens opsonized with C3 fragments that induces phagocytosis, and thereby clearance, of these pathogens. As such, CRIg functions as a key mediator of the first line of host defence against systemic infections.

Karen Honey

ORIGINAL RESEARCH PAPER Helmey, K. Y. *et al.* CRIg: a macrophage complement receptor required for phagocytosis of circulating pathogens. *Cell* **124**, 915–927 (2006)

IMMUNOTHERAPY

Right place, right time

The delivery of biological therapy to the right place at the right time is a key aim for the treatment of cancer. Such directed therapies will have an enormous advantage over non-specific chemotherapeutic approaches that do not differentiate between cancer cells and non-cancer cells. Reporting in *Science*, Thorne *et al.* have found a way of combining two candidate antitumour approaches to achieve specific targeting of tumours and to increase efficacy in mouse tumour models compared with either approach alone.

The authors isolated a population of cells — which they named cytokine-induced killer (CIK) cells — that are derived from human peripheral blood or mouse splenocytes after culturing in the presence of interferon-γ, interleukin-2 and CD3-specific antibody. The CIK cells express T-cell markers and the natural killer (NK)-cell receptor NKG2D (NK group 2, member D), through which they recognize and kill cells expressing the stress-associated ligands MHC-class-I-polypeptide-related sequence A (MICA) and MICB. Because MICA and MICB are expressed under conditions of cellular stress, such as in the tumour microenvironment and after viral infection, CIK cells can be used to specifically target tumour cells *in vivo* despite not knowing any tumour-specific antigens.

To further increase the cytolytic capabilities of CIK cells, the authors infected them with a modified vaccinia virus. The key advantage of this modified vaccinia virus is that it has been engineered to preferentially replicate in and lyse transformed cells. This was achieved by deletion of the viral genes encoding thymidine kinase and viral growth factor. This modified virus, referred to as double-deleted vaccinia virus (vvDD), only replicated in dividing cells in which cellular thymidine kinase expression is upregulated and in cells with dysregulated growth-factor-receptor signalling (both common features of tumour cells).

Putting these two approaches together therefore provides a means to target tumour cells and deliver a cytolytic agent in a synergistic manner. The synergy is due, in part, to the replication of vaccinia virus in CIK cells being delayed for more than 48 hours after infection, allowing the CIK cells time to reach the tumour site before releasing the lytic virus.

The authors used non-invasive bioluminescence imaging to track vvDD-infected CIK cells following transfer to mice bearing tumours. Importantly, two days after intravenous transfer, vvDD-infected CIK cells were detected at the tumour site and were barely detectable in other parts of the body. Moreover, vvDD-infected CIK cells were shown to penetrate deeper into the tumour mass than vvDD delivered alone.

Next, the authors tested the antitumour efficacy of the combination therapy compared with each therapy alone. Injection of immunodeficient mice bearing established human peritoneal tumour xenografts with CIK cells alone could extend the survival of the mice by 8 days. Injection of vvDD alone increased survival by 15–16 days. By contrast, the combination therapy resulted in a complete response in all mice with no relapse for the duration of the study (90 days). This efficacy of the combination therapy was also seen in immunocompetent mice bearing mouse breast cancers. In this case, injection of vvDD-infected mouse CIK cells resulted in complete responses in six of eight mice, whereas injection of mouse CIK cells and vvDD separately (but on the same day) resulted in a complete response in only one of eight mice.

Although the animal models are encouraging, further studies will be required to determine whether this combination approach will translate to humans.

Lucy Bird

ORIGINAL RESEARCH PAPER Thorne, S. H., Negrin, R. S. & Contag, C. H. Synergistic antitumor effects of immune cell-viral biotherapy. *Science* **311**, 1780–1784 (2006)