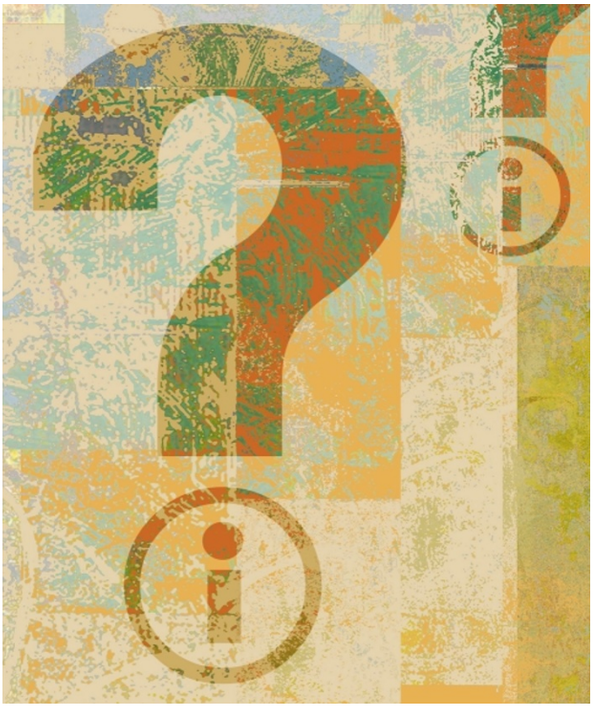
HAEMATOPOIESIS

Stem cell origins



Haematopoiesis is the process whereby mature blood cells of distinct lineages are produced from mulitpotent haematopoietic stem cells (HSCs). Despite decades of study, the origin of these HSCs during mammalian embryogenesis has remained unknown. Reporting in *Immunity*, Cumano and colleagues show that, in mice, HSCs are generated in an intraembryonic region, termed the splanchnopleura (Sp), and not from the extraembryonic yolk sac (YS) blood islands.

During vertebrate embryogenesis, haematopoietic cells first appear within the extraembryonic YS. As the bone marrow and fetal liver (the main haematopoietic organs in mammals) require an input of exogenous haematopoietic precursors to generate differentiated progeny, it was suggested that HSCs originate in the YS and later migrate to the fetal liver. However, the intraembryonic Sp region has also been shown to have haematopoietic activity.

The differentiation potential of haematopoietic precursors from the YS and Sp, separated from mouse embryos before circulation, has been investigated previously by the authors. These experiments established that lymphoid potential is restricted to precursors of intraembryonic origin. In transplantation experiments using normal, irradiated mice as recipients, neither precursors from the YS or from Sp were able to reconstitute an adult haematopoietic compartment. As this might have been due to technical limitations of the system used, the authors developed a new organ culture and cell-transfer system to investigate this further. The protocol involved culturing the Sp and YS, again separated before the onset of blood circulation, for 4 days (to ensure a sufficient number of HSCs were available), before transplanting the precursors into recombinase activating gene (*Rag*)$^{-/-}$ or *Rag*$^{-/-}$ crossed with common γ-chain (*Rag*γ*c*$^{-/-}$) mice. *Rag*$^{-/-}$ mice lack T and B cells (so reducing possible competition between donor and recipient precursors) and *Rag*γ*c*$^{-/-}$ mice additionally lack natural killer cells (which might kill haematopoietic precursors).

NATURAL KILLER CELLS

Natural killer selection

Natural killer (NK) cells are often called 'innate lymphocytes' as they combine innate recognition with the effector mechanisms of T lymphocytes — cytokine secretion (interferon-γ; IFN-γ) and cytotoxic killing. However, in terms of responses to infection, NK cells might be more similar to lymphocytes than previously thought. A report from Dokun *et al.* in *Nature Immunology* indicates that, similar to T and B cells, antigen-specific NK cells selectively expand in response to infection.

NK cells identify their targets by integrating signals from their inhibitory and activation receptors. But not all NK cells are created equal. Although their antigen receptors are invariant, NK cell subsets carry distinct complements of receptors. Previously, this group showed that, in mice, an activation receptor of the Ly49 family, Ly49H, confers specific protection against murine cytomegalovirus (MCMV). This implies that NK cells are capable of virus-specific recognition.

So, are Ly49H$^+$ NK cells preferentially activated by MCMV? The present study assessed this in two ways — by scoring for IFN-γ production through intracellular cytokine staining and by measuring NK cell proliferation using a bromodeoxyuridine (BrdU)-incorporation assay. In the early phase of the response to MCMV there is a burst of IFN-γ production by NK cells, which peaks at 36 hours. However, at 2 days post-infection, there are comparable percentages of Ly49H$^+$ and Ly49H$^-$ NK cells producing IFN-γ, and there was no difference in the proliferation of the two subsets. By contrast, the authors found that by day 6 post-infection, there has been an outgrowth of Ly49H$^+$ NK cells, and these cells proliferate to a much greater degree than their Ly49H$^-$ counterparts.

But is this preferential proliferation actually driven by Ly49H recognition of MCMV? Vaccinia virus infection, which induces NK cell activation, did not cause the selective proliferation of Ly49H$^+$ cells, indicating this might indeed be an MCMV-specific response. In addition, administration of Ly49H antibodies was shown to inhibit bulk NK cell expansion in response to MCMV, which shows that Ly49H has a direct role in MCMV-triggered NK cell activation.

This study provides a new model of NK cell activity in viral infection. Yet it remains to be seen whether this delayed, preferential proliferation of NK cells is itself important for antiviral immunity.

Jen Bell

References and links

ORIGINAL RESEARCH PAPER Dokun, A. O. *et al*. Specific and nonspecific NK cell activation during virus infection. *Nature Immunol.* **2**, 951–956 (2001)

FURTHER READING Cerwenka, A. & Lanier, L. L. Natural killer cells, viruses and cancer. *Nature Rev. Immunol.* **1**, 41–49 (2001)

WEB SITE

Wayne Yokoyama's lab: http://www.hhmi.org/research/investigators/yokoyama.htm

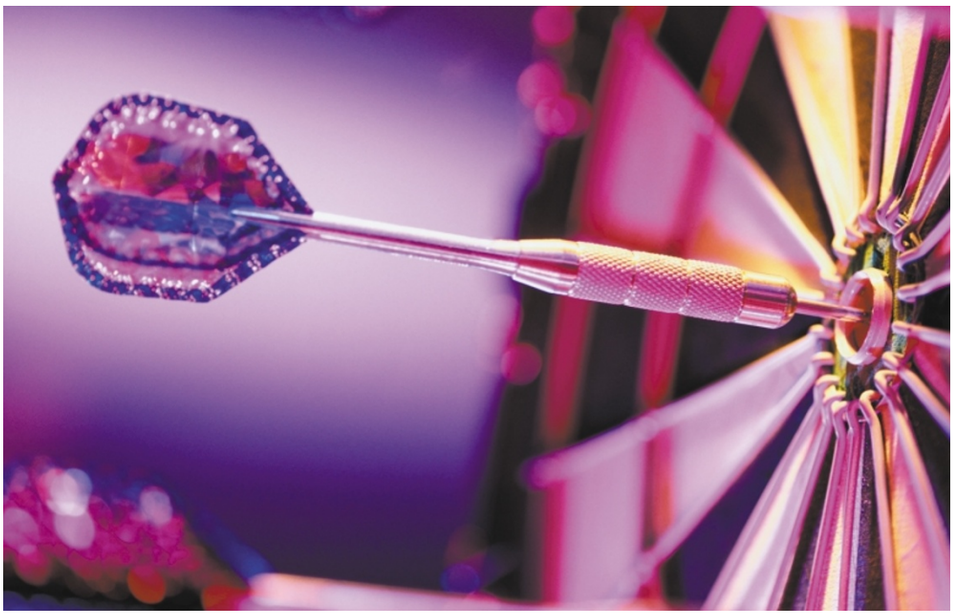
*Rag*γ*c*$^{-/-}$ mice injected with YS cells generated donor-derived myeloid cells, but this reconstitution was transient, and after 3 months donor-derived progeny were no longer observed. No lymphocytes were ever detected in these mice. Therefore, YS cells cannot generate lymphocytes, but can provide short-term myeloid reconstitution. By contrast, after injection of Sp-derived cells into *Rag*γ*c*$^{-/-}$ recipients, donor-derived myeloid cells, as well as B and T cells were generated. These myeloid and lymphoid cells were still present 8 months after injection, indicating that Sp-derived percursors can provide long-term reconstitution of recipient mice.

The authors conclude that the only cells capable of adult long-term haematopoiesis are from the Sp region. So, during mouse embryogenesis, HSCs are generated intra-embryonically and do not derive from the YS.

Jenny Buckland

References and links

ORIGINAL RESEARCH PAPER Cumano, A. *et al.* Intraembryonic, but not yolk sac hematopoietic precursors, isolated before circulation, provide long-term multilineage reconstitution. *Immunity* **15**, 477–485 (2001)



NATURAL KILLER CELLS

Targeting tumour cells

The 'missing-self' hypothesis, formulated by Klaus Kärre and colleagues in 1986, proposed that natural killer (NK) cells seek out and destroy cells that have lost expression of major histocompatibility complex (MHC) class I antigens. Two recent papers, published in *Nature* and in *Proceedings of the National Academy of Sciences*, now show that NK cells can reject tumour cells that express ligands for the activating NK receptor NKG2D, despite the expression of MHC class I molecules by the tumour cells.

The formulation of the missing-self hypothesis predicted the existence of receptors on NK cells that inhibit their activity and which recognize MHC class I molecules. Many of these receptors have now been identified. Recent work has also identified several activating NK receptors, including the lectin-like molecule NKG2D, whose engagement provides dominant activating signals to the NK cell. Previous work by Tom Spies' group showed that NK cells can kill NKG2D ligand-expressing cells *in vitro*. The mouse ligands for NKG2D are retinoic acid early inducible-1 (Rae-1) and H60, which are expressed by some tumour cells, but not by normal adult cells.

Both groups looked for direct evidence to support the idea that tumour cells ectopically expressing ligands for NKG2D could stimulate antitumour responses by NK cells. The Raulet group used a retroviral expression system to express Rae1β and H60 in three mouse tumour cell lines that express MHC class I molecules — EL4 thymoma cells, RMA T-cell lymphoma cells (which were used in the original Kärre study) and B16-BL6 melanoma cells. Transduced EL4 and B16-BL6 cells that were injected subcutaneously into recipient mice were rapidly and completely rejected. Tumour cells were rejected in wild-type mice depleted of CD8$^+$ T cells and in *Rag*$^{-/-}$ mice (which lack T and B cells), but grew in *Rag*$^{-/-}$ mice depleted of NK cells, indicating that conventional NK cells are responsible for the rejection. Rae1β- or H60-transduced RMA cells were also rejected in wild-type mice, but rejection required both CD8$^+$ T cells and NK cells.

The Lanier group also used the RMA cells to investigate NK cell responses. RMA cells stably transfected with Rae1γ or Rae1δ were injected intraperitoneally into recipient mice. Mice injected with mock-transfected cells developed tumours and died, whereas mice challenged with transfected cells rejected tumour cells in an NK-cell dependent manner.

So, NK cells can reject tumour cells expressing NKG2D ligands, despite MHC class I expression. But do mice primed with NKG2D ligand-expressing tumour cells develop an adaptive T-cell response against subsequent challenge by non-transduced parental tumour cells? This is where the results from the two groups differ. Raulet and colleagues found that NKG2D ligand-negative tumour cells were rejected by mice previously challenged with transduced tumour cells, and that this was a CD8$^+$ T-cell-dependent process. By contrast, Lanier and colleagues found that mice that had rejected Rae1γ-transfected RMA cells were unable to reject parental tumours on re-challenge.

These results show that NK cells can, and do, participate in rejection of MHC class-I bearing tumour cells and, although there are some discrepancies in the results, this approach might be effective for tumour vaccine development.

Elaine Bell

References and links

ORIGINAL RESEARCH PAPERS Diefenbach, A., Jensen, E. R., Jamieson, A. M. & Raulet, D. H. Rae1 and H60 ligands of the NKG2D receptor stimulate tumour immunity. *Nature* **413**, 165–171 (2001) | Cerwenka, A., Baron, J. L. & Lanier L. L. Ectopic expression of retinoic acid early inducible-1 gene (*RAE-1*) permits natural killer cell-mediated rejection of a MHC class I-bearing tumor *in vivo*. *Proc. Natl Acad. Sci. USA* **98**, 11521–11526 (2001)

WEB SITES

Lewis Lanier's lab: http://cc.ucsf.edu/people/lanier_lewis.html

David Raulet's lab: http://mcb.berkeley.edu/labs/raulet/