be considered an early event in the cascade leading to necrotizing vasculitis. In the kidneys of the Wistar-Kyoto rats that were used in this experimental model, crescentic necrotizing lesions develop similar to those of human ANCA-associated glomerulonephritis, but it is unknown if hemorrhage precedes these lesions in the kidney. It could be argued that “glomerular hemorrhage” does occur in human ANCA-associated glomerulonephritis. The figure shows examples of this phenomenon in a patient with anti-MPO antibodies in a glomerulus that is normal (top left), in one with a beginning crescent (top right), and in one with chronic changes of the Bowman capsule in the presence of extracapillary proliferation (bottom left). The leaking erythrocytes are transported by the tubules (bottom right), leading to erythrocyturia. However, this is a phenomenon occurring in many renal diseases, only some of which are known to be mediated by autoantibodies. In conclusion, Little and colleagues have elegantly demonstrated that ANCA-ameliorate leukocyte adhesion, transmigration, and hemorrhage in vivo. It is now time to further explore the link between these processes and the development of the necrotizing vasculitic lesion.

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New data suggest that a mild preparative regimen of antibodies that block CD40 ligand and deplete host NK cells may make allogeneic hematopoietic stem cell transplants safe, establish long-term immunologic tolerance, and broaden the applicability of cord blood as a source of stem cells by making engraftment more efficient.

In Greek mythology, “chimera,” derived from the Greek word for “billy goat,” referred to a fire-breathing she-demon that was part lion, part goat, and part dragon or snake. Getting rid of this killer was quite an achievement, and Bellerophon was heaped with praise and riches for his courageous and clever dispatch of the beast. The term “chimera” has come down to us through the ages with 3 definitions: the original monster, an impossible and fanciful creation of the imagination (eg, Woody Allen’s malefactor with the body of a crab and the head of social worker), and an organism containing tissues from at least 2 genetically distinct parents. It is this last definition that is of compelling medical interest, based largely on the work of Ray Owen.

In 1945, Dr Owen was the first to demonstrate immunologic chimeraism, when he found that the majority of dizygotic bovine twins had identical blood types. This chimeraism was thought to result from blood (and, by inference, hematopoietic stem cell) mixing through placental vascular anastomoses. The implied immunologic tolerance associated with the condition was formally documented in 1952 by Billingham et al, who showed that dizygotic chimeric twin cattle were tolerant to skin grafts from each other but rapidly rejected third-party grafts.

Chimerism and its associated immunologic tolerance are easy to induce in fetuses and neonates but difficult to induce in adults with their own immunologic integrity. Many barriers exist to engraftment of donor lymphoid and hematopoietic tissues in adult hosts, but most of these barriers can be overcome by eliminating or greatly suppressing host immune defenses. Clinically, this elimination is usually accomplished by a cytotoxic preparative regimen that usually involves drugs but occasionally also uses radiation therapy. However, these regimens generally do not eliminate natural killer (NK) cells or NK activity. The more recent exploration of nonmyeloablatative preparative regimens has chiefly sought to block the host T-cell response but has largely ignored NK activity. But since the work of Gustavo Cudkowicz (see, for example, Cudkowicz and Stimpfling) in the 1960s, we have known that donor marrow can be rejected even in the face of major histocompatibility complex (MHC) compatibility, and subsequent work from many groups has confirmed a role for NK cells in this process.

In this issue of Blood, Westerhuis and colleagues demonstrate that immunologic tolerance across a major histocompatibility barrier associated with immunologic chimeraism is greatly facilitated by depleting host NK cells, in this case with anti-NK1.1 monoclonal antibody (see figure). In the experiment shown here, C57BL/6 mice were treated with anti-CD40 ligand antibody (anti-CD154) and given 1 million allogeneic BALB/c bone marrow cells on day 0. At days 23 and 27, mice received either phosphate-buffered saline (PBS), anti-NK1.1 to deplete NK cells, or anti-CD8 to deplete cytotoxic T cells. On day 28, a novel in vivo cytotoxicity assay was performed in which 10 million BALB/c donor splenocytes labeled with carboxyfluorescein succinimidyl ester (CFSE) were administered to the mice intravenously, and the elimination of the cells by the host was followed by flow cytometry on peripheral blood samples 2 days later. The results demonstrate that NK cells mediate elimination of 94% of the donor cells after

**NK cells mediate the elimination of donor cells after anti-CD40L mAb treatment.**
anti–CD40 ligand antibody is used as a non-
myelosuppressive preparative regimen for
allogeneic transplantation. The rejection of
donor type cells is largely prevented by deplet-
ing NK cells. In other experiments, adding
NK cell depletion to the preparative regimen
enhanced allogeneic bone marrow donor cells’
ability to establish stable chimism by at least
3-fold. Without NK cell depletion, an inocu-
lum of 30 million BALB/c marrow cells es-
established donor chimerism in only one of 5
C57BL.6 mice; with NK cell depletion, the
same dose of donor cells was 100% effective at
establishing long-term donor chimism.

This paper is important because it focuses
again on the NK cell as an important barrier in
establishing chimerism and immunologic tol-
erance. Early work demonstrated that host
NK depletion facilitated engraftment and he-
matologic recovery in both syngeneic and allo-
genic bone marrow transplants. This paper
joins others that have supported the idea that
NK cells may be important targets for allograft
engineering. It would seem that the time has
come for a clinical test of the hypothesis that
host NK cell depletion can enhance donor
marrow cell engraftment. Given the magni-
tude of the effect of NK depletion reported
here, if the result were verified in humans,
important applications would include nonmy-
elosuppressive allogeneic transplantation and
cord blood transplantation. In both settings,
the capacity to permit complete hematopoietic
engraftment with lower doses of donor cells
might permit safe allogeneic transplants with a
lower incidence of graft–versus–host disease.

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Comment on Hardtke et al, page 1924

**Where you are is what you are**

**Peter Lane  MRC CENTRE FOR IMMUNE REGULATION**

In this issue, Hardtke and colleagues conclusively demonstrate that expression on T
cells of the B-cell–associated chemokine receptor, CXCR5, is essential for mi-
gration into B follicles (follicular B helper T cells [Tfh]) and, further, that T cells
are excluded from B follicles by the T cells’ expression of the T–zone–associated
chemokine, CCR7.

B and T cells are normally segregated
within lymphoid tissue by chemokine
gradients that are established by local popula-
tions of lymphoid stromal cells. CXCR5+ B
cells are attracted to the areas forming B fol-
cicles, whereas both CCR7+ T cells and anti-
gen-presenting dendritic cells are attracted to
sites that form T zones. This isolation of B
cells from T–cell areas is essential for the pro-
duction of high-affinity antibodies, as it ex-
cludes T cells of irrelevant specificities from
the B follicle in which B-cell selection by “li-
ensed” Tfh occurs.

As others have found,1 induction of
CXCR5 on T cells occurs rapidly following
priming and identifies cells destined to pro-
vide help in B follicles but not inflammatory
responses. Although CXCR5 is expressed on a
fraction of primed T cells, many
such cells continue to coexpress CCR7, an
expression motif that localizes them at the
B–T interface where the chemokine gradients
establishing the B follicle and T zone compete
for influence. Since activated CXCR5–
expressing B cells mirror the situation in T
cells by up-regulating CCR7,2 they too localize
at the B–T interface, an arrangement likely to
optimize the efficiency of B–T collaboration
not only for primary antibody production but
also for memory responses. Only a minority of
CXCR5+ CCR7+–expressing T cells go on to
down-regulate CCR7 and become mature Tfh
where they foster the development of B-cell
germinal centers, the structures within which
affinity maturation of the B-cell response oc-
curs. It is not certain which signals induce T
cells to do this; perhaps the signals arise from
some as-yet-unidentified interaction with an-
tigen-activated B cells.

One very interesting observation from
Hardtke et al is that dual CXCR5+CCR7+–
expressing T cells appear in lymph nodes
where there is no obvious ongoing B-cell
response (see figure), suggesting that these
T cells might have migrated there following
priming elsewhere. This possibility is sup-
ported by the fact that these T cells are
found in blood.3 I think that these are recirc-
ulating memory cells primed to provide
B-cell help and that their dual expression
of CXCR5+CCR7+ guides them to a
CD4+CD3− non–dendritic cell located at the
B–T interface.4 My colleagues and I
have found that the development and persis-
tence of T memory cells that provide help to
B cells depend on OX40 (CD134) and CD30
survival signals from these cells.3 The loca-
tion of the T memory cells at the B–T inter-
face puts them in pole position to provide
rapid help to B cells at the earliest sign of
reinfection, but the theory remains to be
tested.

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Expression of CXCR5 and CCR7 and positioning of T
cells to B-cell follicles in LNs and PPs. See the com-
plete figure in the article beginning on page 1924.
Chimera: from bane to blessing

Dan L. Longo