Comment on Little et al, page 2050

Watching ANCAs work

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Previous in vitro studies have shown that antineutrophil cytoplasmic autoantibodies (ANCAs) affect leukocyte–endothelial interactions, which are considered elementary in the development of necrotizing vasculitis. By intravital microscopy, Little and colleagues were able to study these interactions and their consequences in vivo.

Are antineutrophil cytoplasmic autoantibodies (ANCAs) pathogenic? Recent publications have provided compelling evidence that they are.1 But how do they contribute to the development of the necrotizing vasculitis lesions that are so characteristic of the ANCA-associated vasculitides? In this issue of Blood, Little and colleagues provide evidence that ANCAs enhance leukocyte–vessel wall interactions in a model of Wistar-Kyoto rats that developed experimental autoimmune vasculitis after immunization with human myeloperoxidase (MPO). In these rats, leukocyte–vessel wall interactions (adhesion and transmigration) were studied in mesenteric venules by intravital microscopy, a technique that brings us very close to actually watching the lesions of ANCA-associated vasculitis develop.

The choice of the mesenteric venules for the in vivo investigation of leukocyte–vessel wall interactions was most likely guided by practical purposes, as the venules were not affected by vasculitis. In fact, ANCA-associated mesenteric vasculitis is extremely rare; although it needs to be mentioned that it did occur in one of the first patients described with polyarteritis nodosa, in whom practically every organ was affected by the disease. In their famous case history from 1866, Kussmaul and Maier write that “in the mesentery . . . arterial branches are degenerated and thickened to the highest degree, in parts swollen to countless gray-yellow nodules from millet seed to peasized.”2(p11) In advanced ANCA-associated vasculitis, vessels from practically all organs may become involved, which underlines the statement by Little and colleagues that endothelial dysfunction in systemic vasculitis is global. However, it is remarkable that in limited or early disease, a predilection exists for vasculitis of the kidneys and lungs, which indeed is also the case in the Wistar-Kyoto rats that Little and colleagues used. This brings us to the point of organ specificity: it would be interesting to know which factor prevents the early development of vasculitis in the mesentery or otherwise enhances the development of vasculitis in the kidneys and lungs.

Another interesting point is whether the hemorrhage in the mesenteric vessels should
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 ANCs 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.

**REFERENCES**


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**Comment on Westerhuis et al, page 2215**

**Chimera: from bane to blessing**

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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 chimerism, when he found that the majority of dizygotic bovine twins had identical blood types. This chimerism 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 nonmyeloablative 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 Stimpfling3) 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 chimerism is greatly facilitated by depleting host NK cells, in this case with anti-NK1.1 monoclonal antibody (see figure). In the experiment shown here, C57BL6 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