to generate resolving macrophages.[3] Plasmin was also able to increase apoptotic-neutrophil efferocytosis by macrophages, indicating a potentiation of the resolution of the inflammation. Annexin A1 mediated the actions on plasmin-inducing neutrophil apoptosis and efferocytosis (see figure), and in vivo administration of plasmin is able to increase cell surface expression and secretion of annexin A1 by macrophages.[1] Annexin A1 is a potent anti-inflammatory effector molecule of the resolution of inflammation, being one of the main mediators of glucocorticoid anti-inflammatory actions, partially by mediating apoptosis and clearance of apoptotic neutrophils.[10] The work of Sugimoto et al suggests that resolving inflammation through modulation of the plasmin system could represent an advantageous therapy with fewer side effects than the use of glucocorticoids.

This study proposes the plasmin system as an important effector in establishing an efficient resolution of the inflammatory process, paving the way for further studies in the years ahead to test the pharmacological modulation of the plasmin system to achieve an efficient resolution of inflammation. This will ultimately affect the development of novel therapies for a wide range of chronic degenerative diseases with an inflammatory base that affect an increasing elderly population in developed countries.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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● ● ● RED CELLS, IRON, AND ERYTHROPOIESIS

Comment on Zaimoku et al, page 2908

Immune insights into AA

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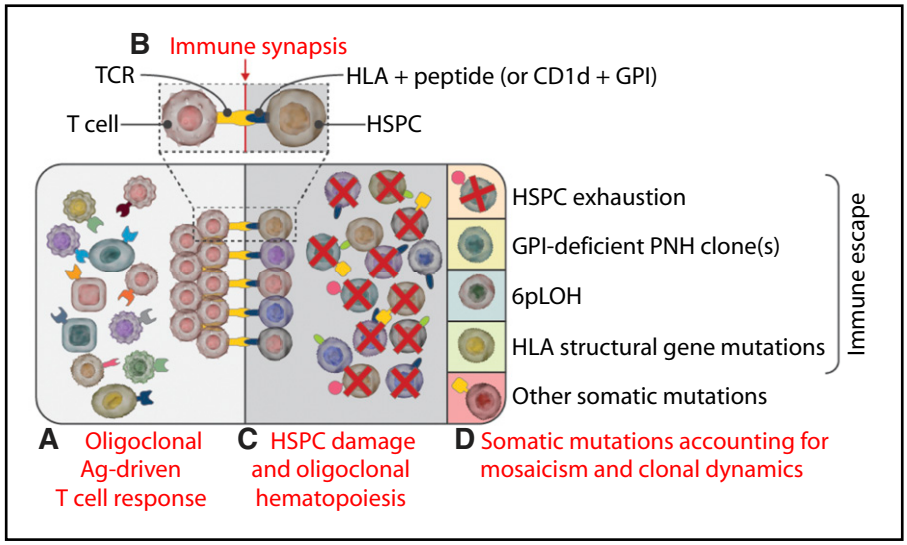
In this issue of *Blood*, Zaimoku et al demonstrate that the functional loss of the HLA-B4002 allele is common in aplastic anemia (AA) patients, suggesting that this allele plays a major role in the immune attack underlying the pathophysiology of this disease.[1]

The immune-mediated pathophysiology of AA is substantiated by the excellent clinical response to immunosuppressive treatment, and supported by a plethora of experimental studies.[2] Most studies suggest that AA is attributable to a T-cell–mediated immune attack targeting hematopoietic stem/progenitor cells (HSPCs). Indeed, different groups have documented in AA the presence of an oligoclonal T-cell response in vivo, with cytotoxic activity of these T-cell clones on autologous HSPCs in vitro.[3,4] However, although T-cell repertoire oligoclonality suggests the presence of an antigen-driven T-cell response, the identification of putative autoantigen(s) triggering such immune response remains elusive. Different HLA alleles were found associated with AA, including *DRB1*1501*, *DRB1*1502*, *B*5201*, and *B*4002*.[5] Furthermore, neutral copy-number loss of heterozygosity of the short arm of the chromosome 6 (6pLOH) emerged as a relatively common phenomenon in AA,[5] suggesting the hypothesis that it may represent a mechanism of immune escape for HSPCs.

In this work, Zaimoku et al confirm that *HLA-B*4002* is among the HLA alleles most frequently carried by AA patients, and that 6pLOH is particularly common in these *HLA-B*4002* AA patients.[5] Using an ultrasensitive flow cytometry assay exploiting a new anti-HLA-B4002 monoclonal antibody, Zaimoku et al demonstrate that HLA-B4002$^-$ granulocytes can be found not only in all *HLA-B*4002* patients with a 6pLOH, but also in the majority of patients without 6pLOH. Indeed, deep sequencing of *HLA-B*4002* in sorted HLA-B4002$^-$ granulocytes isolated from these AA patients without 6pLOH documents that the loss of HLA-B4002 was because of somatic mutations in the *HLA-B*4002* gene, leading to the specific phenotype of HLA-B4002$^-$A$^+$ granulocytes, which cannot be defined as 6pLOH. These HLA-B4002$^-$A$^+$ granulocytes were detected also in AA patients with 6pLOH, leading to the conclusion that HLA-B4002$^-$ granulocytes in these patients are a mosaic of cells truly carrying 6pLOH (HLA-B4002$^-$A$^-$) and cells lacking HLA-B4002 because of other structural gene mutations. Indeed, the authors were able to identify different *HLA-B*4002* somatic mutations leading to a loss-of-function phenotype. In addition, in the same patients a few missense mutations were found in phenotypically normal (HLA-B4002$^+$) granulocytes. All these observations suggest that these HLA-B4002$^-$ cells tend to expand as a result of continuous immune pressure from which they are spared.

The concept of possible immune escape in the context of AA is not a novel concept in bone marrow failure, since it was first introduced by Rotoli and Luzzatto to explain the pathophysiology of clonal expansion of glycosylphosphatidylinositol (GPI)–deficient cells in paroxysmal nocturnal hemoglobinuria (PNH).[6] Autoreactive T cells would target normal HSPCs via some GPI-linked protein or via the GPI anchor itself, eventually sparing



Somatic mutations, hematopoietic mosaicism, and clonal dynamics in immune-mediated AA. (A) From the polyclonal T-cell repertoire, some clonal T cells specific for some antigen expressed on HSPCs (see "Immune synapsis") may expand, leading to an oligoclonal antigen-driven T-cell response. (B) The immune synapsis: T cells may recognize through their T-cell receptor–specific antigens, presented on (some) HSPCs within either HLA alleles (peptidic epitopes) or HLA-like molecules (for lipidic epitopes; this is the case as with the GPI anchor presented within CD1d).[6,7] (C) Pathogenic T-cell clones may exert T-cell–mediated cytotoxicity over many HSPCs (via the immune synapsis depicted in the inset), eventually leading to oligoclonal hematopoiesis.[2,4] (D) Different somatic mutations may stochastically occur within individual HSPCs; because of the underlying HSPC oligoclonality, any neutral mutation carried by surviving HSPCs becomes evident (Darwinian selection).[9] Individual mutations leading to specific functional phenotypes shape the subsequent hematopoietic mosaicism and clonal dynamics through different mechanisms, including immune escape, HSPC fitness, or proliferative advantage. In the absence of somatic mutations, HSPCs may undergo exhaustion (first quadrant). Expansion of clones escaping the immune response may occur through different mechanisms, such as GPI-deficient cells (PNH, second quadrant)[6] or functional loss of HLA due to 6pLOH (third quadrant) or to other structural HLA gene mutations (ie, B4002$^-$ cells).[1] Other somatic mutations may contribute to clonal dominance through distinct specific mechanisms (fifth quadrant)[10]: true malignant transformation for splicing genes, survival/growth advantage, or increased HSPC fitness for epigenetic mutations; unknown (possibly immune escape?) for *BCOR-BCORL1* mutations. Ag, antigen; TCR, T-cell receptor. Professional illustration by Somersault18:24.

PNH (GPI-deficient) HSPCs.[6] CD1d-restricted, GPI-specific T cells were found with a higher frequency in PNH patients, eventually suggesting that an immune attack targeting the nonpeptidic GPI anchor could account for the expansion of PNH hematopoiesis.[7] More recently, the same CD1d-restricted, GPI-specific T cells have been found increased in AA patients, suggesting that the GPI anchor itself may serve as the target antigen even in the autoimmune process underlying AA.[8] With their new study, Zaimoku et al show that immune escape in AA may occur also as a result of HLA loss, eventually providing evidence that HLA-restricted antigen(s) may play a role in AA pathophysiology. These novel findings only appear to contradict their previous data,[8] because in an autoimmune process like AA, the oligoclonal T-cell response may target different antigens, eventually shaping the clonal dynamic of residual hematopoiesis. In this context, HLA-B4002 seems to have a major role in the presentation of some typical, still unknown peptidic antigens, while CD1d does the same for the glycolipidic GPI anchor. This noncasual role of HLA-B4002 is also supported by the observation that in presence of effective immunosuppressive therapy the aberrant HLA-B4002$^-$ cells may reduce as a result of dilution from a restored, phenotypically normal, polyclonal hematopoiesis.[1] However, because Zaimoku et al did not perform the same deep investigation on other HLA alleles, it is not clear whether this propensity to somatic mutations and subsequent functional immune selection is specific of *HLA-B*4002*, or rather it is a broader phenomenon eventually pertaining to any HLA allele.

Recent studies exploiting next-generation sequencing have documented the presence of mutations within different genes frequently involved in myeloid malignancies.[9] The work by Zaimoku et al demonstrated a surprisingly high rate of mutations in the *HLA-B*4002* gene, supporting an underlying autoimmune attack rather than indicating a propensity to progress toward myeloid malignancies. Unfortunately, this study could not formally investigate the mutation rate in AA HSPCs nor any possible hotspot mutation within the HLA locus. However, the finding described in this article may lead to some speculations (see figure): (1) somatic mutations are frequently detectable in AA, possibly as a result of Darwinian selection (ie, most mutations are neutral, and they emerge simply because of oligoclonal hematopoiesis)[9]; (2) some somatic mutations may eventually lead to clonal expansion because of an immune privilege (ie, the so-called immune escape, which is well established for PNH cells[6] and now also for HLA-B4002 cells[1]; it might be hypothesized also for *BCOR/BCOR-L* mutations); and (3) the causal role of other somatic mutations in terms of possible malignant transformation requires further demonstration (ie, mutations in genes responsible of epigenetic regulation may simply affect clonal dominance, whereas mutations in splicing genes are more likely to confer a malignant phenotype).[10]

In conclusion, the data from Zaimoku et al spotlights the role of HLA-B4002 in the autoimmune pathophysiology of AA. This allele is involved in the recognition and subsequent damage of normal HSPCs through the presentation of the causative autoantigen and the formation of the immune synapsis required for T-cell–mediated cytotoxicity. Although further studies are needed to identify the candidate antigen(s) causing AA, current data confirm the immune pathophysiology of AA and allow a better understanding of somatic mutations, hematopoietic mosaicism, and clonal dynamics in the context of immune-mediated bone marrow failure syndromes.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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TRANSPLANTATION

Comment on Hoenig et al, page 2928

HCT for SCID: one size does not fit all

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In this issue of *Blood*, Hoeing et al further our understanding on the clinical presentation and treatment of reticular dysgenesis (RD), a rare entity of the severe combined immunodeficiency diseases (SCIDs).[1] The SCIDs are phenotypically and genotypically heterogeneous, and RD is considered 1 of the most severe forms.[2,3] RD is inherited as an autosomal recessive disorder and accounts for <2% of SCID and classed as T-, B-, and natural killer–deficient SCID.[2] Clinically, it is characterized by an absence of granulocytes and lymphocytes in peripheral blood, hypoplasia of the thymus and secondary lymphoid organs, lack of innate and adaptive humoral and cellular functions, and sensorineural hearing deficit.[3] Unlike classical SCID, RD presents very early, usually within a few days after birth, and with life-threatening bacterial infections rather than opportunistic infections. A complete blood count with differential should alert the astute clinician to consider SCID in the differential diagnosis and undertake urgent consultation with an immunologist. Children with RD have mutations in both copies of the adenylate kinase 2 gene[3] (see figure). The resulting defect in mitochondrial adenylate kinase 2 results in defective maturation of lymphoid and myeloid cells. Consequently, hematopoietic cell transplantation (HCT) is the only treatment curative for this otherwise fatal disease.

In describing the natural history of the disease, Hoenig et al note the high proportion of premature births, infants small for gestational age, life-threatening infections much earlier than seen with classical SCID, lymphopenia, and agranulocytosis. Other hematological features observed in their cohort included thrombocytopenia and hemoglobin levels below the normal range. The most common finding on examination of bone marrow morphology was arrest of myeloid differentiation at the promyelocytic stage. The majority of infants presented within the first week after birth. The authors recommend a diagnostic workup for infants with unexplained leukopenia because early referral to a specialist and HCT is potentially lifesaving. Although long-term survival after HCT was 68%, graft failure or persistence/recurrence of agranulocytosis was the predominant cause of treatment failure.[1] Myeloablative transplant conditioning regimens and transplantation of T-cell–replete grafts were associated with best outcomes.[1]

This is an important observation because transplant strategies for classical SCID vary. For classical SCID, grafts from HLA-matched siblings or from unrelated donors are unmodified and recipients receive immunosuppression posttransplant for graft-versus-host disease prophylaxis.[4] Grafts from HLA-mismatched relatives are T-cell–depleted and administered without further immunosuppression for graft-versus-host disease.[4,5] Although the majority of HLA-matched and mismatched related donor transplants occur without a transplant-conditioning regimen, recipients of unrelated donor transplants receive transplant-conditioning regimens that are more likely to be reduced in their intensity.[4] For several other primary immunodeficiency diseases, reduced intensity conditioning regimens with alkylating agents result in sustained engraftment and long-term survival.[6] Given the rarity of RD, we have to conclude that myeloablative regimens and transplantation of T-cell–replete grafts are preferred to reduced intensity conditioning regimens. Although RD is a SCID, it is associated with other hematopoietic abnormalities, primarily agranulocytosis; this is likely why myeloablative transplant conditioning regimens are needed to ensure sustained engraftment of the transplanted hematopoietic cells. However, the available data do not allow for recommending specific myeloablative regimen(s).

Timely referral is critical because donor search, donor workup, and procurement of graft typically takes 6 to 12 weeks depending on donor source (ie, longer times are needed for adult unrelated donors). Another, often overlooked, aspect of transplantation associated with survival and graft failure is donor selection. An unaffected HLA-matched sibling, when available, is the "gold standard." Selecting unrelated adult donors who are HLA-matched to their recipient at the allele level at HLA-A, -B, -C, and -DRB1 results in the best survival and lowest rate of graft failure for nonmalignant diseases.[7] A similar approach should be considered when selecting umbilical cord blood units; better matched units are associated with lower rates of graft failure.[8] Mismatched related donor HCT, until recently, has been associated with graft manipulation (T-cell depletion) to overcome the HLA barrier. The relatively new approach of using posttransplant cyclophosphamide with a T-cell–replete graft may be an acceptable alternative. However, one must be cautious in adopting strategies that are tested for marrow failure for primary immunodeficiency diseases.[9,10]

Although it is tempting to recommend phase 2 trials to study optimal transplant conditioning regimens, it is not feasible for

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Immune insights into AA

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