fail to fully suppress virus replication; (3) development of functional T-cell exhaustion that is associated with increased expression of specific T-cell inhibitory molecules; (4) establishment of a state of chronic, generalized immune activation that predicts the progression to AIDS; and (5) ineffective T-cell regeneration with impaired function of the thymus and bone marrow and disruption of lymph node architecture. The pathophysiologic role of CD4+ T-cell responses is particularly ambiguous, as these responses may protect against both HIV and opportunistic pathogens and at the same time provide additional targets for virus infection. As the inhibition of virus replication induced by ART leads to a significant reversion of the HIV-associated abnormalities described above and a major reduction in morbidity and mortality, the development of IRIS represents the paradoxical clinical complication of an overall better functioning immune system (see figure). Conceivably, the immune system reconstitution induced by ART, with the accompanying rapid reactivation and expansion of pre-existing but previously depleted and/or exhausted CD4+ T-cell clones specific for subclinical or formerly controlled infections may result in poorly regulated immune responses that, for reasons that we do not fully understand, take a marked pro-inflammatory, tissue-damaging flavor. In this context, the observation of vigorous, poly-functional, and predominantly effector-memory CD4+ T cells specific for certain opportunistic pathogens in IRIS patients may define a subset of immune cells responsible for this phenotype.

However, many questions regarding the immunopathogenesis of IRIS remain unanswered. First and foremost, what is the precise mechanistic role of pathogen-specific CD4+ T cells, and what other innate and adaptive immune cell types contribute to the IRIS-associated proinflammatory state? Second, what distinguishes the chronic immune activation of the natural history of HIV infection, which is usually reduced by ART, from the immune activation that is typical of IRIS? Third, what sort of pre-existing immune abnormalities confer an increased risk of developing IRIS after initiation of ART? Fourth, to what extent is this expansion of effector memory CD4+ T cells related to the HIV-associated depletion of central memory CD4+ T cells that is known to be a key factor in AIDS pathogenesis? Fifth, what are the similarities and differences in the pathogenesis of the various clinical manifestations of IRIS that occur in association with different pathogens? Ultimately, it is our hope that further insight into the pathogenesis of IRIS will allow for the development of novel immune-based interventions that can prevent and treat this potentially serious clinical complication of HIV-infected patients starting ART.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

REFERENCES

CLINICAL TRIALS

Comment on Powell et al, page 3031

Longer FVIII: the 4th generation

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In this issue of Blood, Powell and colleagues present the first human data after the infusion of a new recombinant factor VIII (FVIII) product that when fused with the Fc fragment of IgG1 results in significantly prolonged half-life in the circulation.

The advent of safe and effective concentrates has meant that a person with hemophilia born today can expect an almost normal life expectancy and minimal morbidity. However, this depends on the early and lifelong use of prophylactic treatment with concentrate and the absence of an alloantibody to FVIII (inhibitor). Prophylaxis with FVIII is hindered by the inconvenience of frequent intravenous administration and cost. The half-life of FVIII is 8 to 12 hours so in hemophilia A prophylaxis is usually administered on alternate days or 3 times a week, which can be challenging especially in individuals with difficult venous access.

Although plasma-derived concentrates are safe and effective, recombinant products are preferred because of their perceived improved safety. March 2012 celebrates the 25th anniversary of the first infusion of recombinant FVIII into a human patient at the University of North Carolina at Chapel Hill. Initially, first-generation recombinant concentrates were formulated with plasma-derived human albumin. The second-generation products no longer contained albumin and the third-generation ones also eliminated human and animal proteins from the final preparation and the manufacturing process. Many manufacturers are now tackling the next step in development, to produce the fourth generation of recombinant FVIII products with a longer half-life. Among the techniques being employed to achieve this are pegylation, polysialylation, and coupling the FVIII to albumin or to the immunoglobulin Fc fragment.

Most serum proteins that are too large for renal filtration have a half-life of approximately 3 days. A notable exception, however, is immunoglobulin G (IgG), which has a half-life of 21 days in humans. This is achieved by binding of the IgG to the neonatal Fc receptor (FcRn) which, despite its name, is expressed throughout life in hepatocytes, the vascular endothelium, and many other tissues. IgG bound to the FcRn during pinocytosis and/or endocytosis is protected from degradation within the lysosomes because at low pH the IgG binds more avidly to the receptor. As the receptor is recirculated to the cell surface the IgG is released at the physiologically neutral pH of the circulation. This process has been...
Gene therapy, an ongoing revolution

Olivier Benveniste  UNIVERSITÉ PIERRE ET MARIE CURIE

In this issue of Blood, Buchlis and colleagues describe the long-term persistence (up to 10 years) of factor IX (FIX) expression in adeno-associated virus serotype 2 (AAV-2)–injected muscles of a patient with hemophilia B.1

This AAV-2 contained a human FIX minigene under the dependence of a cytomegalovirus promoter. The patient had received an intermediate dose (6.0 × 10^11 viral genomes [vg] per kg at several sites of both vastus lateralis muscles) during a dose-escalation trial,2 10 years before his death, which was unrelated to the procedure. Despite evidence of gene transfer and expression 10 months after AAV injection and for the rest of his life, his circulating FIX levels remained subtherapeutic (< 1% of normal), presumably because the injected doses were too low. Nevertheless, this study underlines the fact that, despite potential immune responses to any transgene product, a transferred gene can remain transcriptionally and translationally active for many years with no observable inflammatory infiltration at the injection site.1

Gene therapy may eventually become a realistic option for many monogenic diseases, and 2779 gene therapy studies are currently listed in the ClinicalTrials.gov registry. Several strategies to deliver therapeutic genes have been tried, including direct injection of a plasmid encoding the gene of interest into the target tissue (eg, muscle), which has so far achieved only low transduction efficiency3; and the use of modified viruses to carry the gene to target cells. The first noteworthy success was obtained by ex vivo transduction of CD34+ bone marrow cells with a defective retroviral vector and reinjection into 10 boys with X-linked severe combined immunodeficiency. However, because of insertional mutagenesis, 4 boys developed T-cell acute lymphoblastic leukemia. AAV vectors have evolved over the past decade and now represent a particular interest in vivo gene delivery vehicles. Contrary to retroviral vectors, AAV vectors remain essentially episomal after gene transfer, thus minimizing the issue of insertional mutagenesis. The nonpathogenic nature of AAV vectors is a further advantage. Initially however, AAV vectors could only be produced in small amounts for research purposes and for trials involving few injections and low doses.4-6 Efficient AAV gene transfer has nonetheless been achieved, with some clinical benefit, in Leber congenital amaurosis, for example.7 This bottleneck has now been overcome by the development of

REFERENCES

Comment on Buchlis et al, page 3038

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