Immune Dysregulation in Human Subjects With Heterozygous Germline Mutations in CTLA4

PURPOSE OF THE STUDY. Identify the possible genetic basis among a group of patients presenting with a constellation of findings including hypogammaglobulinemia, CD4 T-cell lymphopenia, autoimmune cytopenias, and lymphocytic infiltration of nonlymphoid organs (brain, lung, gastrointestinal tract).

STUDY POPULATION. A total of 23 patients with varying combinations of the findings noted above were selected from a larger cohort of patients referred to the National Institutes of Health for possible autoimmune lymphoproliferative syndrome (ALPS; based on the presence of lymphadenopathy and autoimmune cytopenias).

METHODS. The patients studied had ALPS ruled out based on functional and genomic studies. Because of their common clinical phenotype, the patients were studied using ex vivo immune function assays as well as evaluated genetically by either whole exome sequencing or selected gene sequencing using the Sanger method.

RESULTS. Six of the 23 patients evaluated from 4 families were found to have heterozygous mutations in the gene encoding the protein cytotoxic lymphocyte antigen (CTLA)-4. The patients carrying these mutations were found to have decreased numbers as well as decreased function of T-regulatory (Treg) cells. In addition, intracellular CTLA-4 expression in Treg cells was diminished in the patient’s cells. Additional findings included hyperproliferation of T cells, a finding that was recapitulated by knocking down CTLA-4 using siRNA in normal cells and abrogated by providing normal CTLA-4 through reconstituting CTLA-4 using vector transfection of patient cells. Finally, patient B cells showed a progressive decline in numbers, an increase in autoreactive cells, and decreased ex vivo proliferation.

CONCLUSIONS. CTLA-4 is a critical immunoregulatory protein for normal T-cell and B-cell homeostasis. In these patients, a single normal allele was insufficient to prevent lymphoid infiltration into the lungs, brain, or gastrointestinal tract or inhibit the development of autoimmune cytopenias. Interestingly, this defect showed incomplete penetrance, and some family members (n = 3) carrying the same mutation as affected patients were either healthy or had only minimal symptoms.

REVIEWER COMMENTS. These patients add to a growing list of newly defined genetic defects that are at the crossroads of immune deficiency and immune dysregulation. In this case, there is evidence that some of the findings, such as the decline in B cells, may progress over time. It is interesting that the clinical findings in these patients actually mirror complications seen with newly designed therapies in cancer immunotherapy aimed at blocking CTLA-4 by using neutralizing monoclonal antibody. It is also possible that the patients with heterozygous CTLA4 mutations could benefit from the use of a CTLA-4 mimetic (CTLA-4-Ig). The finding of incomplete penetrance fits with the findings of a similar phenomenon in other autosomal dominant immune dysregulation syndromes such as ALPS.

AAV-Expressed eCD4-Ig Provides Durable Protection From Multiple SHIV Challenges

PURPOSE OF THE STUDY. Although thousands of individuals become infected daily with HIV, attempts to create a protective vaccine have been unsuccessful. The identification of broadly neutralizing antibodies (bNAbs) from individuals with long-term HIV infection raised the possibility that such antibodies might be generated. Unfortunately, bNAbs are not particularly broadly neutralizing. This article reports the development of an engineered “immunoadhesin protein” with the potential to function as an effective HIV vaccine.

STUDY POPULATION. In vitro studies and rhesus macaque monkeys.

METHODS. In vitro studies were conducted to assess the capacity of candidate protein constructs to neutralize HIV isolates, including those resistant to bNAbs. Subsequently, a candidate construct adapted to rhesus macaque monkeys was cloned into an adeno-associated virus (AAV) vector. This was then used to immunize macaques, which were then challenged intravenously with simian-HIV (SHIV).

RESULTS. Previous work with the imunoadhesin CD4-Ig demonstrated lower affinities than that of bNAbs for HIV envelope, and lower potency in neutralizing HIV isolates. These shortcomings were addressed by the addition of a CCR5 mimetic peptide to CD4-Ig, resulting in a final
construct: CD4 domain-IgG1Fc-CCR5 mimetic or eCD4-lg. Variants of this construct were tested in vitro and shown to neutralize multiple neutralization-resistant virus at low concentrations, micrograms per mL levels, which are probably achievable in humans. Finally, eCD4-lg was adapted to rhesus macaques (RHeCD4-lg), and the gene for the resulting construct was cloned into an AAV vector. This was then administered intermuscularly to 4 macaque monkeys. No adverse effects were observed. These animals and four control animals were challenged intravenously with increasing doses of SHIV. The 4 control animals became infected; the 4 immunized macaques did not. RHeCD4-lg could be measured in the serum of the immunized animals and the serum was effective in neutralizing HIV in vitro. Other studies cited indicated that these protective titers may be maintained for years.

CONCLUSION. An engineered protein that binds to the HIV envelope neutralizes a wide range of HIV isolates. The result was protection of the immunized animals against high-dose intravenous SHIV challenge. This suggests that AAV-delivered eCD4-lg may function as an effective HIV vaccine in humans.

REVIEWER COMMENTS. Why is HIV vaccine development so difficult? The prevention of viral infections, either through immunizations or by previous exposure, is primarily mediated by neutralizing antibodies. HIV evades immune defenses through a variety of mechanisms, including envelope variability that allows virus mutants to escape control by the host immune system, masking of critical regions of the virus envelope proteins by regions that are highly variable but also not critical for the function of the envelope, and viral inhibition of host immune defenses. These factors impair the generation of effective neutralizing antibodies. The current study demonstrates that a different approach may be warranted. The investigators created a protein construct that mimics CD4 and CCR5 interactions with the viral envelope protein. In effect, the engineered protein attaches to and blocks the envelope trimer on the surface of replication-competent HIV particles. The direct administration of such a construct would temporarily result in protection against acute HIV infection, but this would not be practical as a long-term solution for prevention at a population level. However, the investigators were able to “package” the gene for their protein construct into a viral vector that has been used for gene transfer therapy in other areas. There are significant challenges in taking this concept to clinical trials, but the novel approach described in this article may be eventually result in an effective HIV vaccine.

URL: www.pediatrics.org/cgi/doi/10.1542/peds.2015–2776CCCCC

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Pediatrics 2015;136;S277
DOI: 10.1542/peds.2015-2776CCCC

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