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ORIGINAL PRE-CLINICAL SCIENCE

Interleukin-16 deficiency suppresses the development of chronic rejection in murine cardiac transplantation model

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BACKGROUND: IL-16 promotes the recruitment of various cells expressing CD4, a receptor for IL-16. The precise role of IL-16 in transplant rejection remains unknown; therefore, the present study investigated the contribution of IL-16 to the development of chronic rejection in heart transplants.

METHODS: C-H-2^{bm12}KhEg (H-2^{bm12}) donor hearts were transplanted into (1) IL-16-deficient (IL-$16^{-/-}$) C57BL/6J or (b) wild type (WT) control recipients (MHC class II mismatch). Grafts were harvested at 52 days, parenchymal rejection was assessed by the ISHLT grading system, and CAV was examined morphometrically. Graft infiltrating cells were detected 10 and 52 days after transplantation. Intragraft cytokine and chemokine profiles were assessed. To confirm the role of IL-16 in CAV development, C-H-2^{bm12}KhEg (H-2^{bm12}) donor hearts were transplanted into C57BL/6J WT recipients treated with (1) anti-IL-16-neutralization monoclonal antibody or (b) control immunoglobulin G. Grafts were harvested at 52 days, and CAV was quantified morphometrically. Graft-infiltrating cells were examined histologically.

RESULTS: Parenchymal rejection and CAV was significantly attenuated in donor hearts transplanted into IL-$16^{-/-}$ recipient mice compared with WT controls. Donor hearts transplanted into IL-$16^{-/-}$ recipients had a significant reduction in coronary artery luminal occlusion, intima-to-media ratio, and percentage of diseased vessels. CAV was associated with decreased donor organ inflammation, as well as donor organ cytokine (IL-1β and IL-6) and chemokine (MCP-1 and KC) protein expression. Intimal proliferation and inflammatory cell infiltration were significantly reduced in hearts transplanted into recipients treated with an IL-16-neutralization antibody.

CONCLUSIONS: IL-16-deficiency reduced graft inflammatory cell recruitment, and allograft inflammatory cytokine and chemokine production. Therefore, IL-16 neutralization may provide a potential target for novel therapeutic treatment for cardiac allograft rejection.

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Despite advances in surgical technique, donor organ preservation, immunosuppressive agents, and clinical and basic science investigation, cardiac allograft vasculopathy (CAV), or chronic rejection, remains the major limitation to long-term survival in heart transplant recipients. CAV is the leading cause of death in patients who survive more than 1 year after transplantation,[1] with the incidence of angiographically visible CAV exceeding 50% at 5 years.[2] CAV is characterized by intimal proliferation during the early phase of the disease and ultimately manifests as coronary artery stenosis, myocardial ischemia, and subsequent graft failure.[3] Unfortunately, the cellular and molecular mechanisms that underlie the pathogenesis of chronic rejection still remain unknown.

Secreted interleukin-16 (IL-16), which requires CD4 antigen expression for biologic activity, was originally identified as a T-cell chemoattractant factor with specificity for $CD4^+$ T cells.[4–6] Subsequently, IL-16 has also been reported to affect a variety of leukocytes, such as $CD8^+$ T cells, mast cells, dendritic cells, and eosinophils, as well as certain non-immune cells, including fibroblasts, lung epithelial cells, and brain cells.[7,8]

The exact biologic role(s) IL-16 plays in numerous disease states remain controversial. IL-16 has been reported to be potentially both pro-inflammatory and anti-inflammatory in different contexts, in addition to possessing non-inflammatory functions. Skundric et al[9] reported the increased level of IL-16 in the central nervous system fluid of mice during the acute and relapsing phase of experimental autoimmune encephalomyelitis, an animal model of multiple sclerosis and Th1- and Th17-type inflammatory process; treatment with neutralizing anti–IL-16 antibody resulted in diminished $CD4^+$ T-cell infiltration with less demyelination of axons, reversal of paralysis, and a significant decrease in frequency of relapsing disease. In contrast, other investigators have reported that IL-16 reduces CD3-dependent lymphocyte activation and proliferation in vitro.[10] Finally, several additional functions of IL-16 have been identified, including participation in cell motility, cell cycle progression, and up-regulation of monocyte major histocompatibility complex (MHC) class II molecule expression.[11,12]

The role IL-16 plays in transplantation still remains controversial. Furthermore, no studies have examined the effects of IL-16 on heart transplantation or the pathogenesis of chronic organ rejection. Studies have suggested that IL-16 is protective in organ transplantation. Fujita et al[13] reported the immunosuppressive effect of IL-16 on T-cell activation in a human skin transplantation model. Laan et al[14] reported that lung allograft recipients with acute rejection displayed lower bronchoalveolar lavage fluid IL-16 concentrations compared with matched control patients. Conversely, de Oliveira et al[15] reported that higher levels of IL-16 are associated with acute rejection in kidney transplant patients.

The functional activities of IL-16 are plentiful and complicated; therefore, it is difficult to characterize the importance of IL-16 specifically in a process as complex as CAV without being able to study that process in the presence or absence of the cytokine of interest. Accordingly, we investigated the role of IL-16 in the development of chronic allograft rejection using genetically IL-16–deficient C57BL/6 recipient mice.

Materials and methods

All experiments reported in this study were approved by the Stanford University Institutional Animal Care and Use Committee and performed in accordance with the *Guide for the Care and Use of Laboratory Animals.*[16]

Animals

C57BL/6J ($H\text{-}2^b$) (wild type control) and B6.C-$H\text{-}2^{bm12}$KhEg ($H\text{-}2^{bm12}$) male mice were obtained from Jackson Laboratory (Bar Harbor, ME). IL-16 deficient ($IL\text{-}16^{-/-}$) male mice on a C57BL/6 background were generated as previously described.[17] Mice were maintained in the Stanford University animal care facility, which is accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, International. Mice were provided with LabDiet RMH 3000 (non-breeders) or Mouse Diet 5015 (breeders) diet (PMI Nutrition International, St, Louis, MO) and reverse osmosis-filtered water ad libitum.

The $IL16^{-/-}$ colony was monitored for adventitious viral, bacterial and parasitic pathogens by a sentinel mouse program. The sentinels were free of mouse hepatitis virus, mouse rotavirus (EDIM), mouse parvovirus, minute virus of mice, ectromelia virus, Sendai virus, pneumonia virus of mice, respiratory enteric virus III (Reo3), Theiler's murine encephalomyelitis virus, lymphocytic choriomeningitis virus, mouse adenovirus 1 and 2, *Mycoplasma pulmonis, Corynebacterium kutscheri, Streptococcus pneumoniae, Salmonella* spp, *Citrobacter rodentium,* ectoparasites (fur mites, lice), and endoparasites (cestodes, pinworms).

Heterotopic heart transplantation and allograft function analysis

Donor hearts were implanted into the recipient abdomen as described previously.[18] Cardiac allograft survival was evaluated daily by direct abdominal palpation. Cardiac graft function was expressed as a beating score and assessed by the Stanford cardiac surgery graft scoring system, as follows: 0, no contraction; 1, contraction barely palpable; 2, obvious decrease in contraction strength, but still contracting in a coordinated manner, with rhythm disturbance; 3, strong, coordinated beat but noticeable decrease in strength or rate, with distention/stiffness; and 4, strong contraction of both ventricles, regular rate, no enlargement or stiffness.

Anti–IL-16 neutralizing antibody treatment

Anti–IL-16 neutralizing monoclonal antibody (mAb), clone 14.1, was a kind gift provided by Dr William W. Cruikshank, Boston University School of Medicine, Boston, Massachusetts. Treatment doses were based on previous reports, where animals were injected 3 times per week intraperitoneally (IP), 100–200 μg/injection.[19] C57BL/6J ($H\text{-}2^b$) wild-type (WT) mice were injected 3 times/week IP with 200 μg of 14.1 anti–IL-16 neutralizing mAb starting 1 day before transplantation until 52 days after transplant. Control mice received 200 μg isotype-matched mouse immunoglobulin G2a (κ). No other immunosuppression was used.