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Interleukin 5 immunotherapy depletes alloreactive plasma cells

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ABSTRACT

Background: Long-lived plasma cells (PCs) that form after alloantigen sensitization produce donor-specific alloantibodies that generate a positive serum crossmatch and preclude transplantation. New approaches for desensitization, including PC depletion with proteasome inhibition, show promise but carry considerable toxicity. Recently, eosinophils have been shown to govern PC persistence. Interleukin 5 (IL-5) depletion is known to reduce eosinophils in human asthmatics. We hypothesized that treatment with an anti-IL-5 antibody can deplete alloreactive PCs, reduce donor-specific alloantibodies, and serve as a less toxic alternative to proteasome inhibition.

Methods: BALB/c mice were sensitized with B6 skin allografts. Starting at 8 wk after sensitization, control mice received injections of phosphate-buffered saline, whereas experimental mice received weekly injections of an anti-IL-5 antibody. PCs were enumerated by enzyme-linked immunosorbent spot after 8 wk.

Results: All control and experimental recipients of skin allografts developed positive crossmatches when screened at 8 wk after sensitization. All experimental mice treated with anti-IL-5 showed a reduction in their total PC numbers. Also, in contrast to the known adverse effects of proteasome inhibition, experimental mice treated with anti-IL-5 exhibited negligible weight loss or lymphopenia.

Conclusions: Treatment with anti-IL-5 is sufficient to reduce, but not eliminate, alloreactive PCs in the bone marrow. This is because of the targeted reduction of eosinophils leading to a reduction in the PC survival factors a proliferation-inducing ligand and IL-6. Generalized toxicity was not observed in experimental mice. Overall, IL-5 directed immunotherapy can eliminate PC's but is unlikely to be a clinically significant desensitization strategy given the persistence of DSA

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1. Introduction

Approximately 30% of patients awaiting a kidney transplant in the United States have donor-specific antibodies (DSAs) [1]. DSAs can be acquired by previous transplants, blood transfusions, or pregnancy. The presence of DSAs has been associated with hyperacute rejection, antibody-mediated rejection, and high rates of organ loss [2–4]. As a consequence,

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these individuals face longer waiting times for a kidney transplant, with only 6.5% of highly sensitized patients receiving a transplant each year [1].

Plasma cells (PCs) secrete DSAs. These cells are typically long-lived, terminally differentiated, nondividing effector cells that are derived from the B cell lineage [5]. Likely related to this quiescent state, PCs are extremely hardy, and often resistant to attempts at elimination, including radiation [6]. Current desensitization protocols consisting primarily of *intravenous immunoglobulin*, plasmapheresis, and rituximab, either alone or in combination, have achieved some success; however, none of them has consistently reduced DSAs [7–11]. Among various ascribed mechanisms, *intravenous immunoglobulin* and plasmapheresis decrease alloantibody, but fail to directly target the PCs [7,9–11]. Regimens that use rituximab fail to eliminate a large portion of PCs because they do not express cluster of differentiation (CD) 20 [12,13]. Mechanistically, directly targeting PCs should offer an alternative therapy for eliminating DSAs.

We are beginning to understand the signals that govern PC survival. These basic science insights will lead to durable therapies that can directly target PCs for desensitization. New approaches for desensitization, including PC depletion with proteasome inhibition, show promise but carry considerable toxicity. Recently, eosinophils have been shown to govern PC persistence [14]. Interleukin 5 (IL-5) depletion is known to reduce eosinophils in human asthmatics [15,16]. We hypothesized that treatment with an anti-IL-5 antibody can deplete alloreactive PCs, reduce DSAs, and serve as a less toxic alternative to proteasome inhibition in a murine transplant model.

2. Methods

2.1. Treatment of mice

C57BL/6 (B6) and BALB/c mice were purchased from The Jackson Laboratory (Bar Harbor, ME). All animal procedures were carried out in accordance with the Animal Welfare Act. Skin transplantation was performed with the animals under general anesthesia as authorized by the Institutional Animal Care and Use Committee guidelines. During skin transplantation, a round 1- to 1.5-cm-diameter full-thickness graft was harvested after removal of *subcutaneous* fat. The grafts were secured to recipient mice with absorbable suture, and evidence of rejection was tracked with regular inspection. We treated mice thrice weekly with intraperitoneal injections of 100 μg of anti-IL-5 antibody (TRFK5; eBioscience, San Diego, CA).

2.2. Antibodies and flow cytometry

A total of $1–10 \times 10^6$ lymphoid cells were surface stained in 96-well microtiter plates. Flow cytometric analysis was performed with fluorochrome-conjugated monoclonal antibodies to mouse CD4, CD8, anti-F4/80, and Gr1, (eBioscience). B220, CD3, CD4, CD8, IgM, CD19, κ-L chain, λ-L chain, CD11b, IgG1, IgG2a2b, and IgG3 (BD Biosciences, San Jose, CA), live-dead stain (Invitrogen, Carlsbad, CA). FACS analysis was performed using an LSRII and FACS Calibur flow cytometer (BD Biosciences) and the data were analyzed using FlowJo Software (Version 8.8.6; Tree Star, Ashland, OR). Intracellular staining was performed using Fix & Perm (Caltag Laboratories, Burlingame, CA).

2.3. Flow crossmatch

To determine the amount of DSA, flow crossmatch was performed. Allogeneic donor splenocytes were incubated separately with recipient serum at serial dilutions. After incubation with recipient serum, donor lymphocytes were counterstained with anti-B220 and anti-CD3 mAbs, and then incubated with fluorescein isothiocyanate-conjugated rat anti-mouse IgGs: IgG1, IgG2a2b, and IgG3 (BD Biosciences). This technique permitted specific gating on the T and B lymphocyte populations to assess the degree of recipient serum IgG binding to donor-derived lymphocyte, that is, B and T cell crossmatch.

2.4. Enzyme-linked immunosorbent assay

Enzyme-linked immunosorbent assay was used to analyze total serum IgG levels. After cardiac puncture or tail bleed, samples were collected and centrifuged for 10 min at 3200 rpm. Polyvinyl 96-well plates were coated overnight at 4°C with goat monoclonal anti-mouse IgG (reacts with IgG1, IgG2a, IgG2b, and IgG3) (Southern Biotech, Birmingham, Alabama) diluted 1/1000 in phosphate-buffered saline (PBS) plus azide. The plates were washed and blocked with PBS and 1% bovine serum albumin plus azide. Plates were washed and serum samples were diluted in triplicate. Bound antibody was detected using an alkaline phosphatase–conjugated goat anti-mouse IgG (diluted 1/1000 PBS and 1% BSA plus azide; Southern Biotech). Plates were washed and developed using 1 mg/mL of *p*-nitrophenyl phosphate (Southern Biotech) in a developing buffer of 0.1 mol/L of sodium bicarbonate and 1 mmol/L of magnesium chloride (pH 9.8). Absorbances were read at 405 nm using a microplate reader.

2.5. Enzyme-linked immunosorbent spot (ELISPOT)

For quantification of IgG secreting cells, multiscreen HTS plates (Millipore) were coated with anti-IgG (Southern Biotech) in sodium bicarbonate buffer, and then blocked with 2% BSA. Cells were incubated in the plate, undisturbed, for 4–6 h at 37°C and 5.0% CO_2. Anti-IgG–conjugated alkaline phosphatase (Southern Biotech) was then applied. Spots were detected using 5-bromo-4 –chloro-3' indolyphosphate p – toluidine salt/nitro blue tetrazolium chloride (Sigma, St. Louis, MO) and scanned and counted on an ImmunoSpot Analyzer (Cellular Technology Ltd, Shaker Heights, OH).

2.6. Real-time polymerase chain reaction analysis

A total of 10^6 bone marrow cells were harvested and washed with cold PBS. RNA was isolated using QIAshredder and Rneasy Mini Kit (QIAGEN, Valencia, CA) and reverse transcribed using random hexamers and Superscript II reverse transcriptase (Invitrogen). For real-time polymerase chain reaction analysis, TaqMan Universal Master mix (Applied Biosystems, Foster City, CA) was used with primer-probe sets to murine glyceraldehyde 3-phosphate dehydrogenase, a proliferation-inducing ligand (APRIL), and IL-6 (Applied

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