Title
Interleukin-5 - Producing group 2 innate lymphoid cells control eosinophilia induced by interleukin-2 therapy

Permalink
https://escholarship.org/uc/item/9t34x2d3

Journal
Blood, 124(24)

ISSN
0006-4971

Authors
Van Gool, F
Molofsky, AB
Morar, MM
et al.

Publication Date
2014-12-04

DOI
10.1182/blood-2014-07-587493

Peer reviewed
Interleukin-5–producing group 2 innate lymphoid cells control eosinophilia induced by interleukin-2 therapy

Frédéric Van Gool, Ari B. Molofsky, Malika M. Morar, Michelle Rosenzwajg, Hong-Erh Liang, David Klatzmann, Richard M. Locksley and Jeffrey A. Bluestone
Interleukin-5–producing group 2 innate lymphoid cells control eosinophilia induced by interleukin-2 therapy

Frédéric Van Gool,1 Ari B. Molofsky,2,3 Malika M. Morar,1 Michelle Rosenzwajg,4 Hong-Erh Liang,5 David Klatzmann,4 Richard M. Locksley,2,5,6 and Jeffrey A. Bluestone1,2

1Diabetes Center, 2Department of Microbiology, Immunology, and Molecular Genetics, and 3Department of Laboratory Medicine, University of California, San Francisco, San Francisco, CA; 4Assistance Publique – Hôpitaux de Paris, Hôpital Pitíé-Salpêtrière, Biotherapy (CIC-BTi) and Inflammation-Immunopathology-Biotherapy Department (I2B), Paris, France; and 5Howard Hughes Medical Institute and 6Department of Medicine, University of California, San Francisco, San Francisco, CA

Key Points
• Tissue resident group 2 innate lymphoid cells are the main cells producing IL-5 and driving eosinophilia in response to low-dose IL-2 therapy.
• We described a novel cellular network activated during IL-2 treatment that may lead to a more efficient use of IL-2 in immunotherapy.

Introduction

Treatment with interleukin (IL)-2 has been used for more than 2 decades to enhance antitumor immunity in patients with advanced kidney cancer and melanoma.1,2 Unfortunately, this high-dose IL-2 treatment is associated with side effects (ie, capillary leak syndrome and hepatic and renal dysfunction) limiting its clinical utility.3 IL-5 induced eosinophilia is one of the most common and unwanted effects observed in cancer patients treated with IL-2–based therapy.4 Since the discovery of T-regulatory cells (Treg), studies in mice have shown that low-dose IL-2 therapy actually prevents or ameliorates autoimmune diseases by activating and expanding these cells.5,6 These observations were applied in a first series of studies in humans to treat chronic graft-versus-host disease–related vasculitis and hepatitis C virus (HCV)-related vasculitis.1,7-9 These studies showed that low-dose IL-2 treatment could provide clinical benefits for the patient’s disease with minimal side effects.10 However, in a phase I trial in autoimmune type 1 diabetes (T1D), low-dose IL-2 plus sirolimus (an analog of rapamycin) induced a transient reduction of insulin production, suggesting some residual toxicity, possibly due to toxic effects of the drug on pancreatic β-cells and/or to the activation of non-Treg by IL-2 in this setting.11,12

Study design

Mice and cytokine administration

Red5, YetCre13, and ROSA–diphtheria toxin fragment A (DTA) (Gt(Rosa)26DTA) mice were described previously13,14 and injected with IL-2/anti–IL-25 or phosphate-buffered saline (PBS). Mice were maintained in the University of California, San Francisco pathogen-free animal facility in accordance with guidelines established by the Institutional Animal Care and Use Committee and Laboratory Animal Resource Center.

Tissue preparation and flow cytometry

Tissues were processed as previously described and single-cell suspensions were used for flow cytometry analysis with the indicated antibodies.13,14

Clinical studies design and participants

Patient characteristics and studies design for the HCV-related vasculitis and T1D trials have been reported previously.8,15

Results and discussion

IL-5–induced eosinophilia is one of the most common unwanted side effects observed with high-dose IL-2 immunotherapy.4,16,17 To
Figure 1. IL-2 promotes IL-5–producing ILC2s and induces eosinophilia. (A) HCV-induced vasculitis patients received IL-2 at 1.5 million international units (MIU)/day from days 1 to 5 (course 1 [C1]), then at 3 MIU/day from days 15 to 19 (course 2 [C2]), 36 to 40 (course 3 [C3]), and 57 to 61 (course 4 [C4]). IL-5–fold increase (pg/mL) and eosinophil counts in Giga/L were measured just before and after 5 days of IL-2. Normal eosinophil counts in the local laboratory are 0 to 0.7 G/L for men and 0 to 0.5 G/L for women, and are showed as dashed lines. Statistical significance of the differences between the groups was assessed using the Mann-Whitney U test. (B) Correlation between increase in IL-5 and eosinophils for the same patients as in (A). Correlations between eosinophils and IL-5 concentrations were determined by Spearman’s correlation coefficient; \( P = .0218 \). (C) IL-5–fold increase over the time of T1D patients receiving a 5-day course of placebo or IL-2 at doses of 0.33 MIU/day, 1 MIU/day, and 3 MIU/day. (D) Serum IL-5 concentration in Red5 heterozygous mice treated with phosphate-buffered saline (PBS) or IL-2/anti–IL-2 mAb complex administered every other day for 3 doses. (E) Fluorescence-activated cell sorter plots of IL-5–producing cells in the indicated tissues after PBS or IL-2 treatment. (F) Quantitation of CD45+ IL-5+ cells in tissues from IL-5 reporter mice after PBS or IL-2 treatment (VAT: perigonadal VAT). (G) Quantitation of eosinophils (CD45+ CD11b+ SiglecF+) in WT or Rag-deficient (RAG2/2) animals from the indicated tissues after PBS or IL-2/anti–IL-2–treated mice. (H) Fluorescence-activated cell sorter plots of the lineage-defining markers negative subset expressing IL-5 (Red5) in the pancreas after PBS or IL-2 complex (top panel). Characterization of the Red5-producing cells in the pancreas, pre-gated on lineage-negative, CD45+ cells (bottom panels). Mean values ± standard error of the mean. All data were analyzed by comparison of means using unpaired 2-tailed Student's t tests. Data are representative of 3 or more experiments or were pooled from 2 to 3 experiments. * \( P < .05 \); ** \( P < .01 \); *** \( P < .001 \).
evaluate if patients treated with low-dose IL-2 also develop eosinophilia, we used data from 2 clinical trials designed to increase Treg cell numbers and induce peripheral tolerance. In the first trial, 8 10 individuals with HCV-induced vasculitis received 4 courses of low-dose IL-2 injections that induced a significant increase in serum IL-5 with a variable change in eosinophil counts, which moderately increased over normal values in 12 of 89 evaluations (Figure 1A). However, despite variability and a small number of patients, we observed a strong correlation between increased levels of IL-5 and eosinophils in some patients (Figure 1B). Importantly, there was a significant correlation between eosinophil counts and IL-5 plasma levels in those patients that had detectable IL-5 at baseline (Figure 1B; \( P < .02 \)). In the second trial, 15 T1D patients were treated for 5 days with 3 different doses of IL-2. The cytokine therapy induced a transient and dose-dependent increase in plasma IL-5 levels, with a cumulative effect after each injection of IL-2 (Figure 1C). Overall, these data
showed that low-dose IL-2 therapy leads to increased blood concentrations of IL-5 and moderate eosinophilia in some patients. However the mechanism(s) involved in this side effect of the IL-2 therapy was unclear.

To determine the mechanism by which IL-2 treatment induced IL-5 and subsequent eosinophilia, we used a newly generated IL-5 reporter mouse. As in the human studies, analysis of sera showed an increase in IL-5 production after treatment of mice with low-dose IL-2/monoclonal antibody (mAb) complex (Figure 1D). The IL-5+ cells were mainly present in nonlymphoid tissues such as the lung, visceral adipose tissue (VAT), and pancreas, but not in the spleen, suggesting that the major cells producing IL-5 were not typical circulating lymphocytes (Figure 1E). After IL-2/mAb treatment, IL-5+ cell number strongly increased, with an average fourfold to fivefold increase in cell number (Figure 1E-F). Interestingly, in RAG−/− mice, the numbers of IL-5+ cells in the somatic tissues was equivalent or higher to the numbers seen in wild-type (WT) mice (data not shown). Consistent with the IL-5 data, somatic tissues was equivalent or higher to the numbers seen in RAG−/− mice (Figure 1E-F). The IL-5 reporter and deleter mouse model, we provide the first direct evidence that eosinophils induced by IL-2/anti-IL-2 mAb treatment is due to the activation of tissue resident ILC2 resulting in the release of IL-5. Interestingly, under the same condition, blood ILC2s did not accumulate (data not shown). Since peripheral blood mononuclear cells were the only clinical samples available from patients treated with low-dose IL-2, we monitored serum IL-5 concentration and eosinophils counts as a surrogate readout to evaluate the activation of ILC2. The IL-2 therapy led to increased IL-5 levels in sera and eosinophils numbers among the peripheral blood mononuclear cells, suggesting that in addition to targeting Treg, low-dose IL-2 therapy also induces the activation of ILC2 that release IL-5 and drive eosinophilia. However, to directly assess the role of ILC2 in the immunologic outcome and clinical efficacy of IL-2 immunotherapy in humans, more experiments must be conducted where access to tissues is feasible. In conclusion, these observations reveal a novel cellular network activated during IL-2 treatment and provide new information that may lead to a more efficient use of IL-2 in immunotherapy and contribute to a better understanding of the side effect induced by this cytokine in the clinic.

Acknowledgments

The authors thank Michel DuPage for helpful comments on the manuscript, Z.-E. Wang and M. Lee for their technical assistance, and M. Consenzo and D. Fuentes for their animal support. This work was supported by grants from the National Institutes of Health, National Institute of Allergy and Infectious Diseases (AI) and National Institute of Diabetes and Digestive and Kidney Diseases (DK) (AI026918, AI030663, AI078869, HL107202, AI046643, AI107328, K08DK101604, AI102011, AI107328, and DK63720), and grants from the University of California, San Francisco Diabetes Family Fund (A.B.M.), the Department of Laboratory Medicine discretionary fund (A.B.M.), the Sandler Asthma Basic Research Center at the University of California, San Francisco, and the Howard Hughes Medical Institute.

Authorship

Contribution: A.B.M. and F.V.G. designed experiments; A.B.M., F.V.G., M.M.M., and M.R. performed research; A.B.M., F.V.G., Z.-E. Wang and M. Lee for their technical assistance, and M. Consenzo and D. Fuentes for their animal support. This work was supported by grants from the National Institutes of Health, National Institute of Allergy and Infectious Diseases (AI) and National Institute of Diabetes and Digestive and Kidney Diseases (DK) (AI026918, AI030663, AI078869, HL107202, AI046643, AI107328, K08DK101604, AI102011, AI107328, and DK63720), and grants from the University of California, San Francisco Diabetes Family Fund (A.B.M.), the Department of Laboratory Medicine discretionary fund (A.B.M.), the Sandler Asthma Basic Research Center at the University of California, San Francisco, and the Howard Hughes Medical Institute.

Conflict-of-interest disclosure: M.R. and D.K. are inventors of a patent application claiming the use of low-dose IL-2 for treating autoimmune disease, which is owned by their academic institutions and is licensed to ILTOO Pharma in which they hold shares. The remaining authors declare no competing financial interests.

Correspondence: Richard M. Locksley, MS 1032B, Box 0795, 513 Parnassus Ave, San Francisco, CA 94143; e-mail: locksley@medicine.ucsf.edu; and Jeffrey A. Bluestone, S-115 Box 0400, 513 Parnassus Ave, San Francisco, CA 94143; e-mail: jeff.bluestone@ucsf.edu.

References