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Limited T Cell Receptor Diversity Predisposes to Th2 Immunopathology: Involvement of Tregs and Conventional CD4 T Cells

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Autoimmunity or immunodysregulation is often observed in individuals with immunodeficiency. This is particularly true in the context of limited T cell production particularly in “leaky” severe combined immunodeficiencies such as occur with hypomorphic mutations in Rag1, Rag2, Artemis, IL-7R α , and the common gamma chain (γ c) as well as in atypical presentations of Complete Digeorge Syndrome [1,2]. The common phenotypes in patients with these genetic lesions, other than immunodeficiency, are oligoclonal expansion of T-cells, histiocytosis, exfoliative dermatitis and erythroderma, eosinophilia and elevated serum IgE.

A variety of explanations have been proposed for these seemingly paradoxical findings. They have focused on several elements of immune regulation, including a failure of central tolerance due to the abnormal thymic development observed when T-cell production is reduced, and/or to a failure of peripheral tolerance due to a deficiency of regulatory T-cells. It has been proposed that the cause of the immunopathology is the homeostatic proliferation of the remaining T-cells [3,4], although why the immunopathology has a Th2 character remains an enigma.

In an effort to understand the basis for the abnormalities that occur when T cell repertoires are limited, we developed a model in which mice genetically deficient in T cells (Rag2 $^{-/-}$, CD3 $\epsilon^{-/-}$, etc) receive limited numbers of purified CD4 T cells. The transferred cells undergo massive proliferation, which we have termed endogenous proliferation, that appears to be due to recognition of self- or commensal-peptides in the absence of CD4 T cells with overlapping specificity. Recipients of limited numbers of transferred CD4 T cells have as many T cells at equilibrium as mice that received large numbers of CD4 T cells. However, the complexity of the TCR repertoires of mice that received small numbers of cells is very limited. We observed that recipients of limited numbers of CD4 T cells develop severe multiorgan immunopathology, eosinophilia, and, when B cells are present, elevated serum IgE; recipients of large numbers of CD4 T cells have a diverse TCR repertoire and do not develop these abnormalities. Here we present evidence that limited Treg repertoires are critical to the induction of immunopathology and that limited repertoires among conventional T cells are deterministic of the Th2 nature of the immunopathology, based on the “signal strength” hypothesis of CD4 T cell fate determination.

Methods

Mice

B10.A, Ly5.1 B10.A, C57BL/10, C57BL/10 AND TCR Tg, C57BL/10 Rag2 $^{-/-}$, B10.A Rag2 $^{-/-}$, B10.A CD3 $\epsilon^{-/-}$, Ly5.1 C57BL/6, C57BL/6 Rag2 $^{-/-}$, and C57BL/6 IL-4 $^{-/-}$ mice were obtained from the NIAID contract facility at Taconic Farms. C57BL/6 mice were obtained from Jackson Labs. Mice were maintained under specific pathogen-free conditions at the NIAID animal facility.

Adoptive transfer

CD4, CD25+ CD4, CD25- CD4 or CD25-/CD44dull CD4 lymph node cells were obtained by sorting on a FACSVantage SE or FACS Aria (BD). Purities were >99%. In some cases, cells were labeled with CFSE (Molecular Probes) at a final concentration of 1.25 μ M. Cells suspended in PBS were transferred via tail vein into recipient mice.

Flow cytometry

Anti- CD25-APC (PC61), CD4-FITC (3T4), CD44-PE, CD44PE-cy5.5, CD45.1-PE, CD45.2-FITC, CD45.1 PE-cy5, IL-4-PE, IL-5-PE, IFN γ -APC were purchased from BD Pharmingen (San Diego, CA). Anti-FoxP3 was purchased from eBiosciences. Anti-IL-13 (clone 38213) was purchased from R&D and conjugated to APC at the NIAID core custom antibody facility. All flow cytometry was performed on a BD FACSCaliber and analyzed using FloJo software.

Pathology/Immunohistochemistry

Immediately after euthanizing mice in a CO₂ chamber, organs were removed, fixed in neutral buffered formalin and embedded in paraffin. Sections were stained with hematoxylin and eosin. Selected tissues were stained with Masson's trichrome (for collagen) or Luna stain (for eosinophils). Anti-parietal cell antibodies were detected by immunofluorescence on cryostat sections of normal BALB/c stomach. Briefly, sections were treated with 2% fetal bovine serum in 5% dry milk in PBS and then incubated with a 1/50 dilution of serum for 1 hour at room temperature. The presence of autoantibodies was visualized by adding FITC-goat F(ab)₂ anti-mouse Ig (Biosource). Slides were examined under a fluorescence microscope and given a score of 0–4 depending on the extent of parietal cell staining by an observer who did not have knowledge of the treatment the mice had received.

Methacholine hypersensitivity

Airway hyperresponsiveness of control Rag2-/- mice and recipients of cell transfers was measured by challenging the mice with increasing doses of nebulized methylcholine (0, 6 and 12 mg/ml) in PBS and measuring "enhanced pause" using whole body plethysmography (Buxco Electronics) following the manufacturer's instructions. Doses above 12 mg/ml resulted in significant morbidity in compromised mice consistent with asphyxia.

IgE ELISA

Immulon 4 96 wells microtiter plate were coated with 100 μ l/well of antimouse IgE (Pharmingen) at 2 mg/ml in PBS, overnight at 4 degrees. Plates were washed with wash buffer (PBS +0.05% Tween20) and blocked for one hour at room temperature with diluent/blocking buffer (PBS + 0.5% bovine serum albumin (BSA) + 0.05% tween 20). Samples (1/50 or 1/500 dilutions of mouse serum or mouse IgE standard (Pharmingen)) were added and incubated for two hours at room temperature. Plates were washed, and biotin-anti mouse IgE (Pharmingen) was added in diluent/blocking buffer for two hours at room temperature. Plates were washed again and streptavidin-HRP (Pharmingen) (1/4000 dilution in diluent/blocking buffer) was added for one hour. Plates were washed again, TMB solution (Sigma) was added for 2–3 minutes, and Stop Solution (2N H₂SO₄) was then added. Optical densities at 450 nm of the samples were then plotted on the linear part of the standard curve and multiplied by the dilution factor in order to calculate IgE concentration in serum samples.

Results

CD3 ϵ -/- mice received 30,000 or 2 million purified total CD4 T-cells from normal donors. IgE levels were markedly elevated in recipients of 30,000 cells beginning at 8 weeks after transfer. By 12 weeks, IgE levels reached ~100 μ g/ml whereas mice that received 2 million

CD4 T cells did not show increased IgE levels. Transfer of 30,000 cells from which CD44^{bright} and CD25⁺ cells had been depleted caused a further increase in IgE levels, to ~300µg/ml. Recipients of 2 million CD25⁻, CD44^{dull} CD4 cells showed a modest increase in serum IgE concentration at 12 weeks after transfer

Rag 2^{-/-} recipients of 30,000 total CD4 T cells or of CD25⁻, CD44^{dull} CD4 T cells developed a severe pneumonia in which the major infiltrating cells were eosinophils, CD4⁺ T lymphocytes (Figure) and alternatively-activated macrophages, characterized by the presence of microcrystals of the Ym1 protein. These mice had airway hypersensitivity to methacholine, eosinophilic gastritis, and a variety of other organ-specific inflammatory responses. The eosinophilic inflammatory disease occurred in nearly every mouse that had received limited numbers of CD4 T cells but none of the mice that received 2 million total CD4 T-cells displayed these inflammatory responses. Mice that had received 2 million naive T-cells, depleted of memory cells and of Tregs, developed a less generalized and less severe disease about half of the time.

As noted above, in recipients of 30,000 cells, inflammation was not limited to the lung. Eosinophilic gastritis with loss of parietal cells developed. In CD3ε^{-/-} recipients of 30,000 CD4 T cells, serum anti-parietal cell autoantibodies were uniformly present. Eosinophilic esophagitis, and liver inflammation were also observed in mice that received 30,000 CD4 T cells. Of note, colitis, a common feature of lymphopenic mice that receive CD45RB^{hi} T-cells, was only occasionally observed in the recipients of large numbers of CD4 T cells and not at all in recipients of 30,000 CD4 T cells.

Co-transfer of 100,000 polyclonal Tregs with the 30,000 conventional T-cells completely suppressed IgE production at 8 weeks, suggesting that a deficiency in Treg function might account for the Th2 type immunopathology seen in recipients of limited numbers of cells. Although Tregs were present at equal frequency in mice that received either 30,000 or 2 million total CD4 T-cells, it can be anticipated that the TCR diversity of Tregs in recipients of 30,000 cells is considerably less than recipients of 2 million cells.

To test the possibility that limitation in TCR diversity among Tregs was an important factor in the immunopathology that occurred in recipients of limited numbers of cells, Rag2^{-/-} mice were reconstituted with 30,000 or 300,000 cell sorter purified CD25⁺ CD4 Tregs. After 6 weeks, the number of Tregs in the two groups of mice was similar. However, since the number of “founders” was 10-times lower in recipients of 30,000 CD25⁺ cells, it can be assumed that the complexity of their TCRs was lower than recipients of 300,000 CD25⁺ cells. Both groups of mice then received 100,000 CD4⁺ CD25⁺ CD44^{low} T-cells, and pathology was assessed 2–3 months later. Mice that had initially received a large number (300,000) Tregs were far more protected from the eosinophilic disease than those which initially received fewer a small number (30,000) of Tregs.

Discussion

We demonstrate that a limited number of CD4 T-cells that have undergone completely normal thymic development, and have not been depleted of regulatory T-cells, can induce severe Th2-associated multiorgan immunopathology upon transfer into lymphopenic hosts. The Th2 nature of the pathology appears to be dependent on the complexity of the effector cells. The lack of TCR complexity of the regulatory cells is critical for the induction of the immunopathology. There are some similarities in immunopathology to that seen in mouse models of Omenn's syndrome, but there are also some important differences. A Th2 phenotype was observed in the Omenn's models, with Th2 cytokine production and elevated IgE [4]. Homeostatic

proliferation of the few T-cells is certainly a common element in our model as well as the Omenn's model [3].

The mouse models of Omenn's Syndrome differ from our model in that they have abnormal thymic development. They fail to express Aire, suggesting that central tolerance via deletion of organ specific T-cells is impaired, which would not be the case for the cells we transfer into Rag2^{-/-} recipients. Furthermore, generation of Foxp3⁺ T cells was impaired in the Omenn's model [4], where it is normal in our mice. These differences may explain the lack of skin pathology in our model, while it is a prominent feature of both the Omenn's mice and of patients with Omenn's syndrome.

The prominent Th2 phenotype observed in our model and the Omenn's model appears to be linked to the limited repertoire complexity among conventional CD4 T cells. One potential explanation for this is that with the marked reduction in TCR repertoire complexity, it is less likely that for any endogenous or exogenous antigen, a cell expressing a high affinity TCR will be present. Thus, responses will be dominated by low affinity cells that, in normal individuals, would be "out-competed" by cells of higher affinity for given antigens. This lack of competition should allow the low affinity cells, which are stimulated at low signal strength, to develop into Th2 cells. Indeed, the association between low signal strength TCR stimulation and Th2 differentiation is based on studies showing that with a relatively high affinity TCR, Th2 differentiation only occurs when one utilizes the cognate ligand at low concentration or uses modified peptides that bind the receptor with low avidity [5,6]. More recently, we have shown that if one uses a class II tetramer that binds to CD4 cells from a polyclonal, yet antigen-reactive population, those cells that bind with low avidity/affinity to the tetramer preferentially differentiate into Th2 cells when stimulated with a concentration of peptide which normally induces Th1 differentiation in cells with high avidity/affinity (Milner JD, Paul WE, manuscript in preparation). Further support for the signal strength hypothesis is drawn from abnormalities in mice with hypomorphic mutations in proteins in the TCR signaling pathway including ZAP70, LAT and CARMA1 [7–10]. In each case, the mice develop an inflammatory disease with a striking Th2 bias.

Further study of limited diversity/limited signal strength models may help better dissect the varying roles of regulatory and effector elements in these extreme cases of disease, and to give insight in more subtle models of Th2-mediated immunopathology such as allergy and asthma.

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References

1. Milner JD, Fasth A, Etzioni A. Autoimmunity in severe combined immunodeficiency (SCID): Lessons from patients and experimental models. *J Clin Immunol* 2008 May;28(Suppl 1)
2. Markert ML, Alexieff MJ, Li J, et al. Complete DiGeorge syndrome: development of rash, lymphadenopathy, and oligoclonal T cells in 5 cases. *J Allergy Clin Immunol* 2004;113:734–41. [PubMed: 15100681]
3. Khiong K, Murakami M, Kitabayashi C, et al. Homeostatically proliferating CD4 T cells are involved in the pathogenesis of an Omenn syndrome murine model. *J Clin Invest* 2007;117:1270–81. [PubMed: 17476359]
4. Marella U, Poliani PL, Casati A, et al. A hypomorphic R229Q Rag2 mouse mutant recapitulates human Omenn syndrome. *J Clin Invest* 2007;117:1260–69. [PubMed: 17476358]
5. Yamane H, Zhu J, Paul WE. Independent roles for IL-2 and GATA-3 in stimulating naive CD4⁺ T cells to generate a Th2-inducing cytokine environment. *J Exp Med* 2005;202:793–804. [PubMed: 16172258]

6. Brogdon JL, Leitenberg D, Bottomly K. The potency of TCR signaling differentially regulates NFATc/p activity and early IL-4 transcription in naïve CD4+ T cells. *J Immunol* 2002;168:3825–32. [PubMed: 11937535]
7. Siggs OM, Miosge LA, Yates AL, et al. Opposing functions of the T cell receptor kinase ZAP-70 in immunity and tolerance differentially titrate in response to nucleotide substitutions. *Immunity* 2007;27:912–26. [PubMed: 18093540]
8. Jakob T, Köllisch GV, Howaldt M, et al. Novel mouse mutants with primary cellular immunodeficiencies generated by genome-wide mutagenesis. *J Allergy Clin Immunol* 2008;121:179–84. e7. [PubMed: 17767948]Epub 2007 Sept 4
9. Sommers CL, Park CS, Lee J, et al. A LAT mutation that inhibits T cell development yet induces lymphoproliferation. *Science* 2002;296:2040–43. [PubMed: 12065840]
10. Jun JE, Wilson LE, Vinuesa CG, et al. Identifying the MAGUK protein Carma-1 as a central regulator of humoral immune responses and atopy by genomewide mouse mutagenesis. *Immunity* 2003;18:751–62. [PubMed: 12818157]

Fig. 1.
CD4+ immunohistochemistry of lung section from Rag2^{-/-} B6 mouse which had received 30,000 CD4+ B6 T-cells 4 months earlier.