Natural regulatory T cells recognize the heavy constant region (Fc) of immunoglobulins: a novel mechanism for IVIG immunotherapy in Pediatric Immune-mediated diseases

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Kawasaki disease (KD) is a self-limited acute vasculitis of the coronary arteries and the most common cardiovascular disease in children. The causes of KD are unknown.

CD8+ cytotoxic T cells are responsible for the aneurism.

Arterial aneurisms occur as a complication in some patients.

KD is successfully treated by IVIG therapy.
T cell-mediated diseases

- Lack of the appropriate T cell repertoire to clear the pathogen: by-standard T cell activation (i.e. HBV, HCV, etc)

- Mimicry: pathogens share antigens with “self” molecules that perpetuate the inflammation

- Lack of an efficient regulatory T cell (Treg) compartment: Treg are indispensable to downsize pro-inflammatory T cell responses
T cell recruitment: Myofibroblasts play an important role by secreting IL17 in the arterial walls.

Human Pathology 2012
Limitations in the characterization of relevant T cells in KD

1. Tissue-infiltrating lymphocytes are not accessible, only circulating T cells can be studied

2. T cells cannot be expanded and characterized in vitro with specific antigens
Solutions for T cell studies in KD

1. FACS sorting and functional characterization of circulating memory T cells (IL-15r+):
   a) to learn the timing of antigen exposure
   b) to study the functional phenotype of pathogenic T cells

2. T cell cloning (0.5 cells/well) ex vivo to define the dominant T cell functional subtypes:
   a) to learn if the pathogenic T cells are still circulating
   b) to learn if regulatory T cells are already expanded
FACS-sorted memory T cells in acute KD

Autoimmunity 2010
CD4+ Treg clones represent 40% of the T cell clonal phenotype in Acute KD

A. TGFβ

B. IL-10

C. IL-4

D. FOXP3

Autoimmunity 2010
Treg antigen specificity

- **Peripherally induced Treg**: pathogen-specific, they differentiate depending upon antigen dose and homing conditions.

- **Natural Treg**: self proteins, they activate depending upon antigen dose.
Natural Treg specificity after IVIG: nTreg recognition of the heavy constant region (Fc)
Detection of Fc-specific nTreg from PBMC

- PBMC separation by Ficoll Hypaque
- 4 days in vitro stimulation with purified Fc fragments
- Surface staining (anti-CD4 and anti-CD25)
- Intracellular staining (FOXP3)
nTreg expand when stimulated by Fc in vitro
Fc-specific nTreg response in 10 out of 14 sub-acute KD after IVIG
Fc-specific nTregs expansion after IVIG

% CD4+CD25+high T cells

Fc μg/ml

Subject 1  Subject 2  Subject 3  Subject 4  Subject 5

0.0  1.5  3.0  3.0  3.0

No Ag  10  100

Subject 6  Subject 7  Subject 8  Subject 9  Subject 10

0.0  1.5  3.0  3.0  3.0

No Ag  10  100

Subject 11  Subject 12  Subject 13  Subject 14

0.0  1.5  3.0  3.0

No Ag  10  100

P=0.02

% Increase Fc-specific nTregs

Normal  Dilated and Aneurysm
Characterization of Fc-specific nTreg clones from sub-acute KD after IVIG
Fc-specific nTreg clones recognize IgG+ autologous B cells in the absence of exogenous Fc
Fc-specific nTreg recognize processed IgG in a conventional MHC-restricted fashion
Further evidences that the Fc-specific nTreg response is TcR mediated
The expansion of Fc-specific nTreg correlates with suppression of recently activated DR+ CD4 and CD8 but not memory T cells
Fc-specific nTreg expand in a variety of Pediatric inflammatory conditions but not Autoimmunity.
Immature myeloid DC expand and secrete IL-10 after Fc stimulation
Immature mDC
FACS sorted populations

CD11b APC-Cy7
CD11c APC

CD14 PE-Cy7
BDCA-1 PE

IL-10
IL-4

CD86 FITC

72 hours FC stimulated

Immature mDC
FC-specific nTregs expansion in healthy adult donors

Donor 1  Donor 2  Donor 3  Donor 4  Donor 5

CD4+CD25+ Tregs

IL-10 pg/ml
Conclusions

IgG-expressing B cells expand nTreg that recognize the Fc

B cells play a role in T cell homeostasis by expanding Fc-specific nTreg: a new model of B-T cooperation

Fc-specific nTreg operate in the lymph nodes and other lymphoid organs but not in the arteries (CCR7+ CCR6-)

Fc-specific nTreg expansion after IVIG explains why IVIG represents the elective therapy in KD and many Pediatric inflammatory conditions

Fc-specific nTreg should play a critical role in mother–fetus tolerance: fetal thymus is exposed to maternal antibodies