$\gamma\delta$ T Cells in Normal Murine Spleen Assist Immunized $\alpha\beta$ T Cells in the Adoptive Cell Transfer of Contact Sensitivity: Effect of *Bordetella pertussis*, Cyclophosphamide, and Antisuppressor T Cell Monoclonal Antibodies

P.W.	Philip W.	Askenase ^a
W.	Włodzimierz	Ptak ^b
M.	Marion	Szczepanik ^b

^aYale University School of Medicine, New Haven, Conn., USA; and ^bJagiellonian University School of Medicine, Krakow, Poland

Key Words

$\gamma\delta$ T cells
Contact sensitivity
Contrasuppressive
Cyclophosphamide
Suppressor T cells

Correspondence to: Dr. Philip W. Askenase, Section of Allergy and Clinical Immunology, 904 LCI, Yale University, Box 208013, New Haven, CT 06520-8013 (USA)

Our previous studies showed that $\gamma\delta$ T cells, in the lymph nodes and spleen of contact-sensitized mice, were required to assist $\alpha\beta$ T cells in the successful adoptive transfer of contact sensitivity (CS) responsiveness. These $\gamma\delta$ T cell receptor positive (TCR $\gamma\delta$ +) regulatory T cells in immune spleen and lymph node were CD3+, CD4-, CD8+, non-antigen specific, and non-MHC restricted, and preferentially employed V γ 5 and V δ 4 variable-region gene segments.

In new experiments to determine the mechanisms of how $\gamma\delta$ T cells were required to assist $\alpha\beta$ T cells in CS, we found similar regulatory $\gamma\delta$ T cells in the spleen of normal mice, but not in the spleens of nude mice or severe combined immunodeficiency mice, suggesting that (1) the regulatory $\gamma\delta$ T cells were present prior to immunization, (2) they required the thymus for differentiation, and (3) they also required rearrangement of $\gamma\delta$ V gene segments.

Treatment of recipients with *Bordetella pertussis* restored the ability of immune $\alpha\beta$ + $\gamma\delta$ -, T cells, purified using anti-TCR $\gamma\delta$ magnetic beads, to adoptively transfer CS. It was subsequently found that *B. pertussis* caused the mobilization of various cell subsets (including $\gamma\delta$ T cells) from a sessile state in the spleen to an activated circulating state in the blood, and rendered TCR $\alpha\beta$ + CS-effector cells resistant to suppression. Thus, coinciding with the large lymphocytosis following *B. pertussis* administration, the spleen became negative for TCR $\gamma\delta$ + CS-assisting cells, while the peripheral blood cells became positive for this regulatory activity.

Our results suggested that *B. pertussis* caused CS-assisting $\gamma\delta$ T cells to leave the lymphoid organs and enter the circulation, enabling them to assist circulating TCR $\alpha\beta$ + CS effector T cells. Treatment of recipients with a low dose of cyclophosphamide (50 mg/kg) also restored the ability of $\alpha\beta$ + $\gamma\delta$ - T cells to transfer CS. It was thought that treatment with cyclophosphamide ablated or inactivated suppressor T cells in the recipients, that inhibited the adoptive cell transfer

of CS by the immune $\alpha\beta$ T cells. Suppression of CS transfers was also eliminated by treating recipients with two different rat IgM monoclonal antibodies to suppressor T cells.

We concluded that the TCR $\gamma\delta$ + CS-assisting/protecting T cells were contrasuppressor T cells (i.e. could protect TCR $\alpha\beta$ + CS effector T cells from the actions of endogenous suppressor T cells, that normally resisted the adoptive cell transfer of CS). Thus, these non-antigen-specific, non-MHC-restricted, CD3+, CD8+, $\gamma\delta$ T cells that protected TCR $\alpha\beta$ + CS effector T cells, assisted by contrasuppressing the CS effector $\alpha\beta$ T cells, rendering them resistant to suppressor T cells in the normal recipients.

© 1995 S.KargerAG, Basel 1018-2438/95/1073-0363 \$8.00/0