10. VSV-IND and VSV-NJ were supplied by D. Kolakovsky (University of Geneva, Switzerland), and viral titers were analyzed by focus-forming assay. The LCMV-WE virus and the plaque-forming assay were described previously [M. Battegay *et al.*, *J. Virol. Methods* **33**, 191 (1991)]. Vacc-WR plaque-forming assays were performed on BSC40 cells. *Listeria* titers were assessed as described previously [T. Fehr *et al.*, *J. Exp. Med.* **185**, 921 (1997)].
11. The VSV- and LCMV-specific ELISA and the VSV neutralization assay have been described previously [T. Fehr *et al.*, *J. Exp. Med.* **188**, 145 (1998)]. The vacc-WR–specific ELISA was performed similarly.
12. *Listeria* (5×10^6 CFU) were incubated for 30 min with 50 μl of serum of naïve C67BL/6 or μMT mice at different dilutions. Samples were stained with 2 μl of goat anti-mouse IgM^b (PharMingen) for 30 min at 4°C and analyzed with a FACScan. In some samples, 0.1 M α-methyl-D-mannoside was added to block unspecific binding to glycosid groups.
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29. We thank A. Macpherson for sera of germ-free mice and for reading the manuscript. Supported by the Swiss National Science Foundation (grant number 31-50900.97 to R.M.Z.) and the Kanton of Zurich.

16 August 1999; accepted 26 October 1999

The Role of CCR7 in T_H1 and T_H2 Cell Localization and Delivery of B Cell Help in Vivo

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Subsets of murine $CD4^+$ T cells localize to different areas of the spleen after adoptive transfer. Naïve and T helper 1 (T_H1) cells, which express the chemokine receptor CCR7, are home to the periarteriolar lymphoid sheath, whereas activated T_H2 cells, which lack CCR7, form rings at the periphery of the T cell zones near B cell follicles. Retroviral transduction of T_H2 cells with CCR7 forces them to localize in a T_H1-like pattern and inhibits their participation in B cell help in vivo but not in vitro. Thus, differential expression of chemokine receptors results in unique cellular migration patterns that are important for effective immune responses.

Trafficking of cells within secondary lymphoid tissues is carefully orchestrated to ensure that antigen-specific T cells are able to deliver help to antigen-specific B cells (*1*, *2*). Recent studies have shown that chemokines are important in regulating leukocyte trafficking within secondary lymphoid tissues (*3*). Treatment of lymphocytes with pertussis toxin, a potent inhibitor of chemokine receptor signaling, prevents them from entering the splenic white pulp (*4*). The chemokines SLC and ELC, which signal through the chemokine receptor CCR7, and the chemokine BLC, which signals through the chemokine receptor CXCR5, are constitutively expressed in secondary lymphoid tissues and seem to be particularly important in establishing normal lymphoid architecture and trafficking patterns (*5*, *6*). CCR7- and CXCR5-deficient mice have disturbed lymphoid architecture and impaired immune responses, as do mice deficient in SLC and BLC production (*6*–*8*).

$CD4^+$ helper T lymphocyte subsets differ in their abilities to provide B cell help (*9*). T_H2 cells efficiently provide B cell help, promoting strong humoral responses with class switching to immunoglobulin G1 (IgG1) and IgE. T_H1 cells are inefficient at providing B cell help, although they can induce class switching to IgG2a (*10*). In humans, T_H1 and T_H2 cells have also been shown to differ in their repertoires of expressed chemokine receptors (*11*). Human T_H1 cells preferentially express CXCR3 and CCR5, whereas T_H2 cells preferentially express CCR3 and CCR4.

To determine if $CD4^+$ T cell subsets home to different microanatomic locations within secondary lymphoid tissues, we adoptively transferred undifferentiated (naïve) and antigen-stimulated, in vitro–differentiated ovalbumin (OVA)-specific T_H1 and T_H2 cells from DO11.10 transgenic mice into BALB/c recipients and immunized the recipients with OVA (*12*). Two days later, we defined the localization of the transferred cells by immunostaining frozen sections of spleen and popliteal lymph nodes (LNs) (*13*). Transferred T_H1 and naïve cells were concentrated within the periarteriolar lymphoid sheaths (PALS) (Fig. 1, A to C). In contrast, transferred T_H2 cells formed loose rings around the outer PALS in close proximity to the B cell zones. The localization patterns in the spleen were the same with and without antigen immunization. Similar patterns were seen at 1, 4, or 8 days after transfer with the exception that transferred T_H2 cells were difficult to detect by 8 days in vivo (Web figure 1). In the popliteal LN (Fig. 1, D to F), T_H1 and T_H2 cells were both found primarily in the outer cortex in the parafollicular areas. Naïve cells were found in similar locations except that they were recruited in larger numbers and were found throughout the medulla as well. In the absence of local antigen, T_H1 and T_H2 cells were not detected in the LN, and naïve cells were detected in only small numbers. Pretreatment of the T cells with pertussis toxin before transfer disrupted their localization patterns within the spleen and completely prevented migration into the popliteal LN, suggesting a dependence on chemokine receptor signaling (Web figure 2).

Analysis of activated murine T_H1 and T_H2 cell chemokine receptor expression by ribonuclease protection assays and Northern (RNA) blotting (*14*) revealed distinct receptor repertoires in the two populations (Fig. 2, A and B). T_H1 and T_H2 cells expressed similar levels of CCR1, CCR2, and CCR4 mRNA. T_H1 cells preferentially expressed CCR7, CXCR3, and CCR5, whereas T_H2 cells expressed more CCR3 and CXCR4. Neither cell type expressed detectable BLR-1. Analysis of CCR7 expression in naïve

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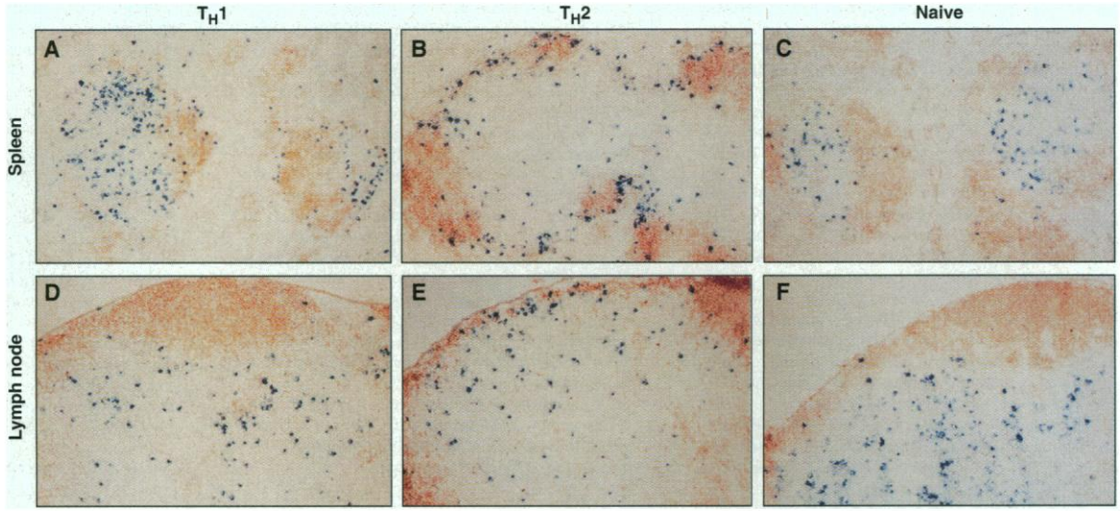

Fig. 1. T_H1, T_H2, and naïve $CD4^+$ T cells display different localization patterns within secondary lymphoid tissues. BALB/c mice received an iv infusion of 2×10^7 T cells, and then were immunized with 100 μg of OVA in incomplete Freund's adjuvant ip (**A** to **C**) or in the footpad (**D** to **F**). Two days later the spleen and popliteal LNs from each mouse were collected. Frozen sections were stained with the clonotypic antibody KJ1-26 (blue) to identify the transferred cells and with anti-B220 (brown) to identify B cell follicles. Shown are spleens (A to C) and LNs (D to F) of recipients of T_H1 cells (A and D), T_H2 cells (B and E), and naïve $CD4^+$ T cells (C and F).

$CD4^+$ T cells showed even higher levels of CCR7 mRNA than in T_H1 cells (*15*). CCR7 expression was unaffected by addition of interleukin-2 (IL-2) to the cultures over a period of up to 14 days (Web figure 3).

Next we tested whether the differences in receptor expression resulted in functional differences by measuring calcium fluxes in response to treatment with chemokines (*16*). T_H1 cells responded to SLC with calcium fluxes in a dose-dependent manner, whereas T_H2 cells failed to respond to SLC at any dose tested up to 300 ng/ml (Fig. 3A). To determine if expression of either CCR7 or CXCR3 was sufficient to confer SLC responsiveness, T_H2 cells were stably transduced with chemokine receptor genes or vector controls by using a retroviral system (*17*) and then tested for SLC responsiveness. T_H2 cells transduced with CCR7 responded to SLC in a manner similar to control T_H1 cells (Fig. 3B). Neither CXCR3-transduced nor control T_H2 cells responded to SLC at the concentrations tested, although transduction with CXCR3 did confer responsiveness to the CXCR3 ligand Mig (*18*).

To determine if CCR7 expression was responsible for the differences in localization patterns between T_H1 and T_H2 cells, we adoptively transferred T_H2 cells transduced with the retrovirus encoding CCR7 or the control retrovirus into wild-type BALB/c mice. As before, the mice were immunized intraperitoneally with OVA, and 2 days later the localization patterns of the transferred cells were analyzed in the recipient spleens. Control transduced T_H2 cells showed the typical T_H2 cell splenic localization pattern, forming rings around the outer PALS near the B cell zones (Fig. 4A). In contrast, T_H2 cells expressing CCR7 behaved in a T_H1-like manner, clustering within the central PALS (Fig. 4B). The effect was CCR7-specific. Transduction of T_H1 cells with CCR3 or CCR8 and transduction of T_H2 cells with CXCR3 had no detectable effect on the microanatomic localization of these cells in the spleen. T_H2 cells expressing BLR-1 formed clusters of cells within the B cell follicles (*19*). T_H1 cells transduced with BLR-1 remained primarily within the central PALS, suggesting that the action of CCR7 is dominant.

The proximity of the antigen-stimulated T_H2 cells to the B cell zones in the spleen together with their known abilities to provide help for B cells suggested that the loss of CCR7

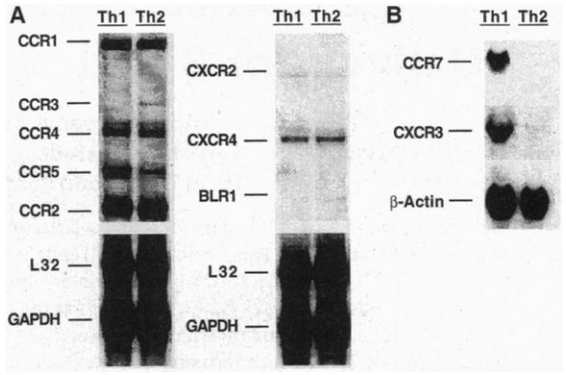

Fig. 2. Murine T_H1 and T_H2 cells differentially express the chemokine receptors CCR3, CCR5, CCR7, and CXCR3. RNA was purified from T_H1 and T_H2 cells 7 days after restimulation with OVA (323–339) peptide. (**A**) Ribonuclease protection assay with 10 μg of RNA from T_H1 or T_H2 cells and the Pharmingen RiboQuant template sets mCR-5 (left) and mCR-6 (right). L32 and GAPDH are housekeeping genes to control for equal loading of the RNA. (**B**) Northern blot analysis. Samples (40 μg) of RNA from T_H1 or T_H2 cells were analyzed with a ^{32}P-labeled CCR7 probe. The membrane was stripped and reprobed for CXCR3 and then stripped and probed again for β-actin as a control for loading.

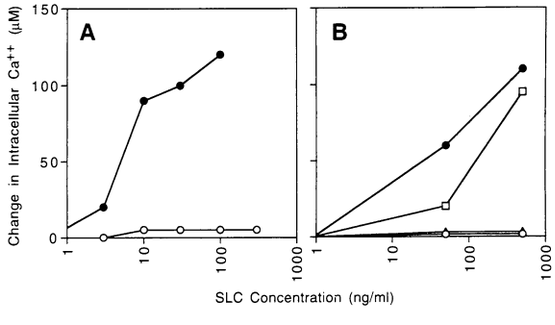

Fig. 3. (**A**) T_H1 cells, but not T_H2 cells, respond to the CCR7 ligand SLC. T_H1 cells (●) or T_H2 cells (○) were loaded with Fura-2 dye and then analyzed by dual-wavelength fluorimetry for increases in intracellular Ca^{2+} concentration in response to SLC. Data are plotted as the change in bulk intracellular Ca^{2+} concentration for a given concentration of SLC. (**B**) Transduction of T_H2 cells with CCR7 but not CXCR3 confers SLC responsiveness. T_H1 or T_H2 cells were transduced with the retroviral vector alone or the vector containing the CCR7 or CXCR3 cDNA. Transduced cells were then loaded with Fura-2 dye and analyzed by fluorimetry for their responsiveness to SLC. Shown are data for T_H1 cells transduced with vector (●), T_H2 cells transduced with vector (○), T_H2 cells transduced with CCR7 (□), and T_H2 cells transduced with CXCR3 (△).

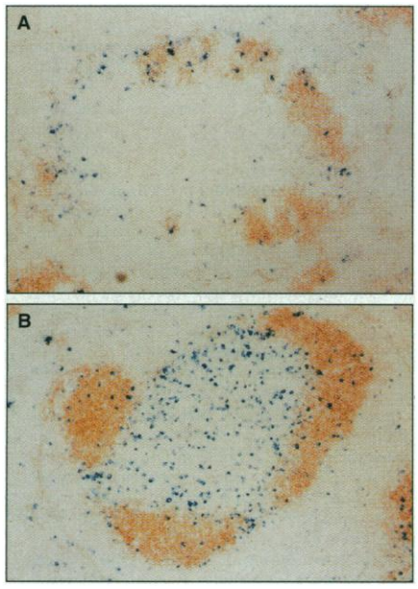

Fig. 4. Forced expression of CCR7 in T_H2 cells results in a T_H1-like splenic localization pattern. T_H2 cells were transduced with the retroviral vector alone (**A**) or with the vector containing the CCR7 cDNA (**B**). Transduced cells (2×10^7) were adoptively transferred into BALB/c recipient mice. Two days later, frozen sections from spleens of recipients were stained with KJ1-26 antibody (blue) to identify the transferred cells and with B220 (brown) to identify the B cell follicles.

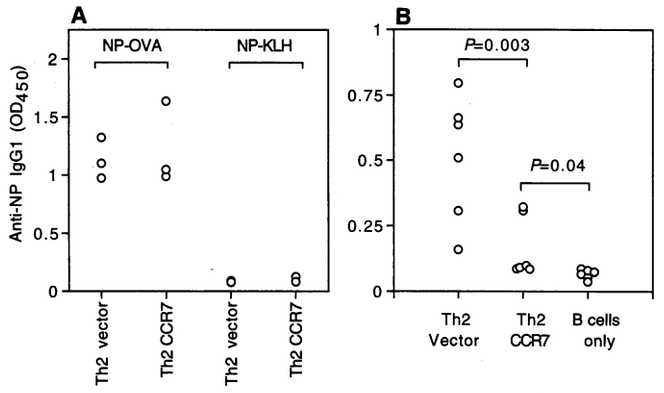

Fig. 5. CCR7 expression impairs the ability of T_H2 cells to help B cells in vivo but not in vitro. (**A**) Evaluation of helper function in vitro. Splenic B cells were purified from mice immunized 14 days earlier with 100 μg of NP-BSA in complete Freund's adjuvant. B cells (1×10^6) were then mixed with 10^5 T_H2 cells that had been transduced with the control retrovirus (T_H2 vector) or with the CCR7-encoding retrovirus (T_H2 CCR7) cells and incubated in a 96-well dish in the presence of either NP-OVA (10 μg/ml) or NP-KLH (10 μg/ml). After 48 hours, the cells were washed and given fresh medium. Five days later the supernatants were collected and anti-NP IgG1 titers were measured by ELISA. (**B**) Evaluation of helper function in vivo. Primed B cells (1×10^7), purified as in (A), were adoptively transferred to irradiated BALB/c mice either alone or mixed with 10^5 T_H2 cells that had been transduced either with the control retrovirus (vector) or with the CCR7-encoding retrovirus (CCR7). The mice were then immunized with 100 μg of NP-OVA adsorbed to alum. Sera were collected 7 days later and analyzed by ELISA for anti-NP IgG1 antibodies. Statistical comparisons were made by Student's *t* test.

expression on differentiated T_H2 cells might be necessary for efficient delivery of B cell help. To test this hypothesis, we transferred B cells purified from NP-BSA [(4-hydroxy-3-nitrophenyl) acetyl–bovine serum albumin]–primed mice into sublethally irradiated naïve recipients together with 10^5 OVA-specific CCR7-expressing or control T_H2 cells (*20*). The recipients were immunized intraperitoneally with NP-OVA, and 7 days later sera were analyzed for anti-NP IgG1 antibodies. In parallel, we cultured a portion of the primed B cells together with the CCR7-expressing or control T_H2 cells in medium containing either NP-OVA or NP-KLH (keyhole limpet hemocyanin). In vitro, where the T cells and B cells were not physically segregated, both CCR7-expressing and control T_H2 cells were effective in providing B cell help when the appropriate antigen, NP-OVA, was present (Fig. 5A). Anti-NP IgG1 was not detected when the cells were cultured with NP-KLH. In contrast, CCR7-expressing T_H2 cells were impaired in their ability to help B cells in vivo, presumably because the forced expression of CCR7 directed these cells to the inner PALS away from the splenic B cell zones (Fig. 5B). Similar results were obtained when CCR7-expressing or control T_H2 cells were transferred to T cell–deficient ($TCR\beta^{-/-}$) mice rather than irradiated wild-type mice (*21*).

CCR7 expression levels, therefore, are critical in determining the location and consequently the function of $CD4^+$ T cell subsets within the spleen. Naïve cells expressing CCR7 are retained in the central PALS (Fig. 1C) near interdigitating dendritic cells that are highly effective at presenting antigen to naïve cells. Antigen-stimulated T_H2 cells, which are effective at promoting humoral responses, lose CCR7 expression and migrate to the peripheral T cell zones in close proximity to the B cell zones (Fig. 1B). Indeed, retention of the T_H2 cells in the PALS by forced expression of CCR7 interrupts delivery of B cell help (Figs. 4 and 5). T_H1 cells, which are poor B cell helpers, maintain CCR7 expression and are retained in the PALS (Fig. 1A). The functional significance of the T_H1 location remains unknown, but it could be important for regulation of cytolytic $CD8^+$ T cells that traffic through the inner PALS (*22*). Also, the cellular requirements for inducing an IgG2a response are unclear. The microanatomic separation between interferon-γ (IFN-γ)–secreting T_H1 cells and B cells in the spleen would seem to prohibit IFN-γ–dependent class switching to IgG2a in this tissue. Interestingly, in the LN both T_H1 and T_H2 cells are in close proximity to the B cell zones, suggesting that there the T_H1 cells may more readily participate in B cell help (Fig. 1, D to F). In the spleen, the location of the T_H1 and T_H2 cells could also influence the type of antigen-presenting cells encountered. $CD11b^{bright}$ dendritic cells of monocyte lineage have been shown to reside primarily in the marginal zones and outer PALS, whereas $CD11b^{dull}$, $CD8^+$ dendritic cells of lymphoid origin are believed to migrate preferentially to the central T cell areas (*23*). Treatment of mice with granulocyte-macrophage colony-stimulating factor, which increases the number of macrophage-related, $CD11b^{bright}$ dendritic cells, increases T_H2 responses. Treatment of mice with Flt3-ligand, which increases the number of lymphoid-related dendritic cells, increases T_H1 responses (*24*). Thus, selected aspects of the spleen microenvironment appear specifically adapted to mediate selected immune responses.

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12. DO11.10 TCR-transgenic mice on a RAG-2–deficient, BALB/c background (>10 generations backcrossed to H-2^d) were provided by O. Kanagawa (Washington University, St. Louis, MO). BALB/c mice were obtained from

Harlan Sprague-Dawley (Indianapolis, IN). All mice used in these experiments were females; 4 to 6 weeks of age and were housed in specific pathogen-free conditions and handled in accordance with institutional guidelines. OVA-specific T_H1 and T_H2 cells were generated in vitro from DO11.10 splenocytes as described [C. S. Hsieh, S. E. Macatonia, A. O'Garra, K. M. Murphy, *J. Exp. Med.* **181**, 713 (1995); D. A. Randolph, C. J. Carruthers, S. J. Szabo, K. M. Murphy, D. D. Chaplin, *J. Immunol.* **162**, 2375 (1999)]. On day 7 after culture with antigen under polarizing conditions, cells were frozen in fetal calf serum containing 10% dimethyl sulfoxide. Before transfer into recipient mice, cells were thawed and restimulated with 0.3 μM OVA (323–339) peptide and irradiated BALB/c splenocytes. Three days later, the cells were diluted 1:4 in medium supplemented with IL-2 (40 U/ml), and used for passive transfer 7 days after thawing. The polarized cytokine profiles of each batch of cells were confirmed by intracellular cytokine staining or by enzyme-linked immunosorbent assay (ELISA) with Quantikine ELISA kits (R&D Systems, Minneapolis, MN). Naïve $CD4^+$ T cells were prepared by generating single-cell suspensions from the LNs and spleens of RAG-2–deficient DO11.10 TCR transgenic mice and centrifuging the cells over a Histopaque-1119 gradient to eliminate erythrocytes and dead cells. The resulting cells were >70% $CD4^+$, and of these >85% were L-selectin$^+$ naïve cells. For passive transfer, 2×10^7 T cells were washed, resuspended in 0.25 ml of phosphate-buffered saline (PBS) and then injected intravenously (iv) into mice that had been anesthetized with Metofane. As indicated, some mice were also injected with OVA (100 μg) in 50 μl of incomplete Freund's adjuvant administered intraperitoneally or subcutaneously in the footpad. In some experiments, T cells were incubated with pertussis toxin (100 ng/ml) for 2 hours at 37°C and then washed five times before transfer.

13. Spleens and LNs were embedded in OCT (Tissue Tek, Elkhart, IN), frozen on dry ice, and then stored at −70°C. Cryosections were cut, air-dried, and fixed in acetone for 10 min. Slides were incubated for 15 min in blocking solution (PBS with 0.5% Tween-20 and 3% normal goat serum) containing avidin (four drops per milliliter; Vector Labs, Burlingame, CA) followed by 15 min in blocking solution containing biotin (four drops per milliliter; Vector Labs). Antibody staining was by incubation for 1 hour in blocking solution containing biotinylated KJ1-26 antibody and fluorescein isothiocyanate (FITC)–B220 (Pharmingen, San Diego, CA). The slides were washed in PBS with 0.5% Tween-20 and then incubated for 20 min in 0.5% H_2O_2 in methanol to quench endogenous peroxidase activity. Color development was for 1 hour with the AP-ABC reagent (Vector Labs) together with horseradish peroxidase–anti-FITC (Sigma). Alkaline phosphatase activity was detected with the Vector Blue Substrate Kit (Vector), and peroxidase activity was detected with Sigma Fast diaminobenzidine substrate (Sigma).
14. RNA was prepared from freshly isolated naïve $CD4^+$ T cells or from T_H1 and T_H2 cells 7 days after initiation of culture under polarizing conditions with guanidine isothiocyanate–CsCl [J. M. Chirgwin, A. E. Przybyla, R. J. MacDonald, W. J. Rutter, *Biochemistry* **18**, 5294 (1979)]. Ribonuclease protection assays were performed with 10 μg of RNA (RiboQuant kit, Pharmingen) with the mCR-5 and mCR-6 template sets. For Northern (RNA) blot analysis, 40-μg samples of RNA were fractionated with 1% agarose gel in formaldehyde and transferred to a nylon membrane (Hybond-N, Amersham Life Science). Gene expression was detected with radiolabeled probes (Multiprime system, Amersham Life Science). For CXCR3, the entire coding region was used as a probe. For CCR7, a 415–base pair Nco I–Hind III fragment was used. Hybridization was at 42°C in 50% formamide and washing was in 0.2× standard saline citrate at 42°C.
15. D. Randolph and D. Chaplin, unpublished data.
16. Cells (2×10^7 per milliliter) were incubated in 3 μM Fura-2 AM (Molecular Probes, Eugene, OR) for 20 min in T cell medium at 37°C in the dark. The suspensions were diluted 10-fold in medium and incubated an additional 20 min, then washed three times in fluorimetry buffer [25 mM Hepes, 1 mM $CaCl_2$, 125 mM NaCl, 5 mM KCl, 1 mM Na_2HPO_4, 0.5 $MgCl_2$, 0.1% glucose, 0.1% BSA (pH 7.4)] and stored on ice until use. The cells were equilibrated briefly at 37°C and then either SLC or Mig (Peprotech, Rocky Hill, NJ) was added. Fluorescence was measured and the average bulk intracellular Ca^{2+} concentrations were calculated with a Hitachi F2000 fluorimeter as described [G. Grynkiewicz, M. Poenie, R. Y. Tsien, *J. Biol. Chem.* **260**, 3440 (1985)]. After the intracellular Ca^{2+} concentration had returned to a stable baseline, 10 μM ionomycin, 0.1% Triton-X 100, and 4 mM EGTA were added sequentially as controls.
17. Chemokine receptor cDNAs were amplified from T_H1 and T_H2 RNA with the Titan One Tube RT-PCR System (Boehringer Mannheim). Forward and reverse primers for CCR7 were GAGACTCGAGAGAGCACCATGGACCCAGG and GAGAGAATTCCTACGGGGAGAAGGTTGTGG and for CXCR3 were GAGACTCGAGATGTACCTTGAGGTTAGTGAACG and GAGAGAATTCGAATTACAAGCCCAGGTAGG (underlined nucleotides designate Xho I or Eco RI sites). Primer sequences for other chemokine receptors tested are available on request. Polymerase chain reaction products were purified by gel electrophoresis, digested with Xho I and Eco RI, and cloned into the Xho I and Eco RI sites of the retroviral plasmid hCD4-RV (provided by T. Murphy, Washington University School of Medicine). hCD4-RV is similar to green fluorescent protein (GFP)–RV as described [S. Ranganath *et al.*, *J. Immunol.* **161**, 3822 (1998)], but with truncated human CD4 as a selectable marker, rather than GFP. Retroviral stocks were prepared with the Phoenix cell packaging line (provided by K. M. Murphy, Washington University School of Medicine) according to the protocol of G. Nolan (Stanford University). Primary T cells from DO11.10/RAG-$2^{-/-}$ mice were activated under polarizing conditions with OVA (323–339) peptide and infected 24 hours later with retroviral supernatants and polybrene (6 μg/ml, Sigma). Six days after activation, transduced cells were purified with FITC–anti-huCD4 followed by anti-FITC MicroBeads on a MiniMACS column (Miltenyi Biotec, Auburn, CA). Purified huCD4-expressing cells were restimulated with OVA peptide and irradiated BALB/c splenocytes and either used directly 7 days later or frozen for subsequent use.
18. Mig has previously been reported to be preferred over SLC as a ligand for CXCR3 [H. Soto *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 8205 (1998)].
19. D. Randolph, G. Huang, D. Chaplin, unpublished data.
20. For measurement of T cell help in vivo, primed B cells were prepared in BALB/c mice by immunization with NP-BSA (100 μg) in complete Freund's adjuvant. Ten days later, splenic B cells were purified with anti-CD43 magnetic beads on a MidiMACS column (Miltenyi Biotec). Purified B cells (1×10^7) were transferred to naïve, sublethally irradiated (400 rads) BALB/c mice either alone or with 10^5 OVA-specific control T_H2 cells or with 10^5 CCR7-transduced T_H2 cells. The recipient mice were immunized ip with NP-OVA (100 μg) adsorbed to 2 mg of alum in 0.5 ml of PBS. Seven days later, anti-NP IgG1 titers were measured in sera by ELISA. For evaluation of helper function in vitro, 10^6 primed B cells were incubated at 37°C in wells on a 96-well plate with 1×10^5 control transduced or CCR7-transduced T_H2 cells in T cell medium containing either NP-OVA (10 μg/ml) or NP–keyhole limpet hemocyanin (10 μg/ml). After 2 days, the medium containing the antigen was removed and replaced with fresh medium. Five days later, culture supernatants were collected and anti-NP IgG1 titers were measured.
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25. We thank T. Murphy and K. Murphy for the retroviral vectors and valuable advice, and O. Kanagawa for the DO11.10/RAG-$2^{-/-}$ mouse strain. Supported by NIH grants T32 GM07200 (to D.A.R.) and AI34580 (to D.D.C.). D.D.C. is an investigator of the Howard Hughes Medical Institute.

27 July 1999; accepted 22 October 1999

Impaired Immunoproteasome Assembly and Immune Responses in *PA28*$^{-/-}$ Mice

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In vitro PA28 binds and activates proteasomes. It is shown here that mice with a disrupted *PA28b* gene lack PA28a and PA28b polypeptides, demonstrating that PA28 functions as a hetero-oligomer in vivo. Processing of antigenic epitopes derived from exogenous or endogenous antigens is altered in *PA28*$^{-/-}$ mice. Cytotoxic T lymphocyte responses are impaired, and assembly of immunoproteasomes is greatly inhibited in mice lacking PA28. These results show that PA28 is necessary for immunoproteasome assembly and is required for efficient antigen processing, thus demonstrating the importance of PA28-mediated proteasome function in immune responses.

Cytotoxic T cells eliminate infected cells by recognizing foreign antigens processed in a proteasome-dependent manner and presented by class I molecules of the major histocompatibility complex (MHC) (*1*). The peptidase activities of the proteasome can be activated in vitro by the interferon-inducible proteasome regulators PA28a and PA28b (*2, 3*). A role for PA28 in MHC class I antigen presentation has been suggested (*3, 4*). However, the underlying mechanism by which PA28 influences antigen processing via a protea-

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