21. K. Yokomori *et al.*, *Genes Dev.* **7**, 2235 (1993).
22. R. D. Klemm *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 5788 (1995); R. Hori, S. Pyo, M. Carey, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 6047 (1995).
23. S. Malik, D. K. Lee, R. G. Roeder, *Mol. Cell. Biol.* **13**, 6253 (1993); Y. Li *et al.*, *Science* **263**, 805 (1994).
24. R. O. Weinzierl, B. D. Dynlacht, R. Tjian, *Nature* **362**, 511 (1993); R. O. Weinzierl *et al.*, *EMBO J.* **12**, 5303 (1993); S. K. Hansen and R. Tjian, *Cell* **82**, 565 (1995); J. L. Chen and R. Tjian, *Methods Enzymol.* **273**, 208 (1996).
25. F. Andel III, A. G. Ladurner, C. Inouye, R. Tjian, E. Nogales, data not shown.
26. We thank R. Glaeser, W. Nicholson, and K. Downing for helpful discussions and O. Arum for help in scanning micrographs. Supported by grants from the NIH General Medical Sciences (E.N.) the NIH Cancer Institute (R.T.), and the U.S. Department of Energy in contract with the University of California (E.N.).

14 September 1999; accepted 2 November 1999

Control of Early Viral and Bacterial Distribution and Disease by Natural Antibodies

Adrian F. Ochsenbein,[1]* Thomas Fehr,[1] Claudia Lutz,[2] Mark Suter,[3] Frank Brombacher,[4] Hans Hengartner,[1] Rolf M. Zinkernagel[1]

Natural antibodies are often dismissed from immunological analysis as "background," but they may play an important role in conferring immunity against infections. In antibody-free mice infected with various viruses or with *Listeria monocytogenes*, viral or bacterial titers in peripheral organs, including the kidney and brain, were 10 to 100 times greater than in antibody-competent mice (and enhanced their susceptibility to some infections), and titers in secondary lymphoid organs were 10 to 100 times lower than in antibody-competent mice. Thus, natural antibodies play a crucial role by preventing pathogen dissemination to vital organs and by improving immunogenicity through enhanced antigen-trapping in secondary lymphoid organs.

The humoral immunity of naïve animals consists of B cells secreting "natural" antibodies representing the spontaneous repertoire of circulating immunoglobulins (Ig's) that mainly belong to the IgM class, although IgG and IgA natural antibodies have also been described (*1*). A major source of natural antibodies seems to be the peritoneal $CD5^+$ B-1 cell subset (*2*). Circulating antibodies against toxins, bacteria, and erythrocytes in the sera of naïve animals were described very early in the history of immunology (*3*). Nevertheless, despite their abundance and early detection, their role, particularly in host protection, remains unclear. Natural antibodies may form part of innate immunity (*4*); may facilitate antigen uptake, processing, and presentation by B lymphocytes via complement and Fc-receptors (*4*); may induce or prevent autoimmune diseases (*1*, *5*); may clear lipopolysaccharides (*6*) and protect against *Streptococcus pneumoniae* (*7*); or may be involved in a hypothetical immunoregulatory idiotype–anti-idiotype network (*8*).

In many virological and immunological assays it is a convenient practice to predilute sera to variable extents, from 1:10 up to 1:80, to avoid so called "nonspecific background" signals; the consequence of this practice is that naturally occurring antibodies are not normally detected or are not registered.

It is recognized that during infections with many cytopathic viruses (such as polio, influenza, and rabies viruses), the presence of early neutralizing antibodies is essential for protection against lethal disease, which often correlates with viral replication in neural tissues (*9*). The possible role of natural antibodies in viral infections has largely remained unexplored. We found a key role of natural antibodies during generalized infections with vesicular stomatitis virus (VSV), lymphocytic choriomeningitis virus (LCMV), vaccinia virus (vacc-WR), or *Listeria monocytogenes*.

Sera from naïve mice assessed by enzyme-linked immunosorbent assays (ELISAs) on purified LCMV- or vaccinia virus–coated plates (*10*) revealed LCMV-specific natural IgM, but not IgG, at a titer of 1:16 (Fig. 1A) or vaccinia-specific natural IgM at a titer of 1:64 (Fig. 1B). LCMV-specific natural antibodies did not detectably neutralize the virus in vitro (*11*), and only LCMV-binding natural antibodies could be assessed (Table 1). *Listeria*-specific natural antibodies were assessed by fluorescence-activated cell sorting (FACS) analysis (Fig. 1C) (*12*); specific natural IgM, but not IgG, antibodies were detectable up to a dilution of 1:16. Sera from naïve C57BL/6 mice were tested for antibodies specific for two different VSV serotypes: VSV–New Jersey (VSV-NJ) and VSV-Indiana (VSV-IND) (*10*). Because VSV glycoprotein (VSV-G) exhibits the neutralizing determinant at its tip, ELISAs with intact VSV measure essentially neutralizing antibodies (Fig. 1, D and E). In all cases, only IgM was detected. Natural G-binding (neutralizing) antibodies to intact VSV-IND did not cross-react with intact VSV-NJ, as shown by the fact that pre-

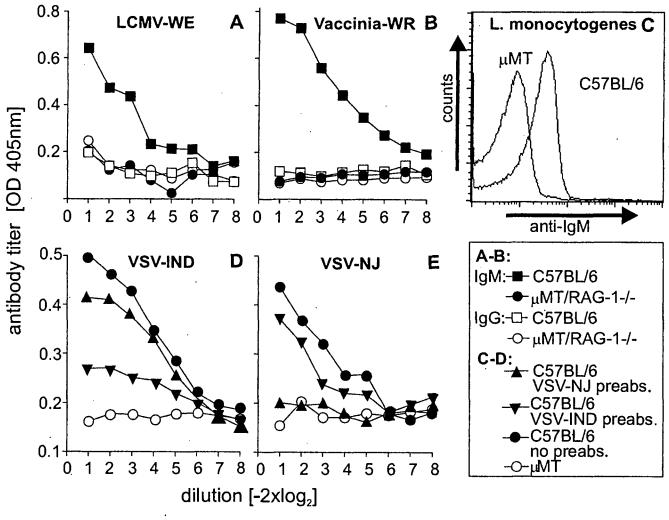

Fig. 1. Detection of natural antibodies. Sera from unimmunized mice were tested in an ELISA (*11*) on purified LCMV-coated (**A**) or purified vacc-WR–coated (**B**) plates for the presence of specific IgM or IgG. (**C**) *Listeria* (5×10^6 CFU) was incubated with 50 μl of prediluted sera (in 1:2 dilution steps; only the 1:4 dilution is shown) from naïve C57BL/6 or μMT mice, stained with fluorescein isothiocyanate–labeled goat anti-mouse IgM^b (from PharMingen) and analyzed by FACS (*12*). Sera of SPF mice were preabsorbed with equal volumes of purified intact VSV-IND or VSV-NJ (≈10^{11} PFU/ml) overnight (**D** and **E**) Thereafter, sera were tested either on plates coated with purified VSV-IND (D) or with VSV-NJ (E) virus particles. The mean of three animals per group is shown; variations in the groups were within plus or minus one titer step of 1:2.

[1]Institute for Experimental Immunology, University Hospital Zurich, Schmelzbergstrasse 12, 8091 Zurich, Switzerland. [2]Max Planck Institute for Immunobiology, Freiburg, Germany. [3]M. Suter, Institute for Virology, University of Zurich, Switzerland. [4]F. Brombacher, Department of Immunology, Groote Schuur Hospital, University of Cape Town, 7925 Cape Town, South Africa.

*To whom correspondence should be addressed.

absorption of sera with VSV-IND reduced only VSV-IND–specific, not VSV-NJ–specific, natural antibody titers and vice versa (Fig. 1, D and E).

Sera from naïve, conventional, specific pathogen–free (SPF) and germ-free (GF) C57BL/6 mice protected in vitro against lytic infection with VSV-IND and VSV-NJ up to a dilution of 1:16 to 1:32 (Table 1), regardless of the hygiene status of the mice. To assess the role of self antigens on natural antibodies, neutralizing natural antibodies were measured in sera of naïve mice that express the glycoprotein of VSV-IND as a transgene under the control of the H-2K^b promotor (KINDG mice) but nevertheless possess VSV-IND–specific B cells that can be induced by infection with VSV (*13*). KINDG mice exhibited no VSV-IND–specific antibodies but showed normal levels of natural VSV-NJ–specific antibodies (Table 1). Neutralization of the virus by natural antibodies was always mediated by IgM, because reduction with 0.1 mM β-mercaptoethanol almost completely inhibited neutralization. Thus, (i) VSV-specific natural antibodies are serotype-specific in vivo, and (ii) low levels of antibodies are produced by VSV-specific B cells independently of intentional stimulation by external or self antigens.

The influence of natural antibodies on early infection kinetics was tested either in mice totally lacking antibody production (μMT and RAG-1$^{-/-}$ mice) (*14*) or in mice that lacked only IgM but did produce all other Ig subclasses (IgM$^{-/-}$) (*15*). We studied virus dissemination between 2 and 8 hours after infection (with the exception of the slowly replicating LCMV, because virus was not detectable in peripheral organs before 24 hours after infection), because this time interval was too short for a specific IgM antibody response to be induced. After infection with 2×10^6 plaque-forming units (PFU) of LCMV-WE, the early trapping of virus particles to the spleen in mice producing antibody was more efficient than in μMT animals (Fig. 2A). The reduced early recruitment of virus to the spleen led to virus titers in the blood and peripheral organs at 24 hours after infection that were up to 10 to 50 times greater than those in antibody-producing mice (Fig. 2B). Infection with 5×10^7 PFU of vaccinia virus or 2×10^7 colony-forming units (CFU) of *Listeria* revealed a comparable effect of natural antibodies on the early dissemination of these pathogens (Table 2).

The role of natural antibodies in virus dissemination and disease was analyzed in detail after infection with VSV. Mice deficient in antibody production showed elevated titers in peripheral nonlymphatic organs such as the liver, kidney, and brain but showed reduced titers in the spleen when compared with antibody-competent mice (Fig. 2, C and D, and Table 2). The replication of VSV in vivo is stringently controlled by interferon-α (IFN-α) (*16*); however, comparison of IFN-α/β receptor$^{-/-}$ [A129 (*16*)] mice with IFN-α/β receptor$^{-/-}$/RAG-1$^{-/-}$ (AR129) mice revealed that IFN-α/β was not responsible for the observed titer reduction in the lymphatic organs of antibody-deficient mice. IgM$^{-/-}$ mice (*15*) exhibited higher titers in peripheral nonlymphoid organs but had a titer in the spleen that was two to three times lower as compared to that in control mice, verifying that IgM was largely responsible for the findings (Table 2).

Reconstitution experiments with μMT and RAG-1$^{-/-}$ mice were performed with 0.25 to 1 ml of naïve serum (Fig. 3A). This substitution, as compared with untreated controls, prevented VSV infection of peripheral organs in a dose-dependent manner and increased viral titers in the spleen. Recruitment to the spleen did not reach the levels found in control spleens, however, for at least three possible reasons: First, the spleens of μMT and RAG-1$^{-/-}$ mice are about two to three times smaller than C57BL/6 spleens; second, the disrupted splenic architecture of B cell–deficient mice (*17*) may contribute to a reduced filtration capacity; third, 1 ml of naïve serum reconstitutes natural antibodies only to about 30 to 50% of control levels. Additional experiments showed that complement inactivation with cobra venom factor revealed no obvious influence on natural antibody–dependent virus distribution (Fig. 3B).

Natural antibodies protected against paralytic disease caused by VSV; the median lethal dose (LD_{50}) in μMT mice was found to be around 10^3 PFU of VSV-IND as compared with 10^8 PFU in controls. Enhanced susceptibility to VSV of B cell–depleted (*3*) or of μMT mice versus controls has been observed previously (*18*). To test the effect of natural antibodies on the course of a VSV infection, μMT mice were injected once with 1 ml of naïve serum intravenously (i.v.) and 1 ml of naïve serum intraperitoneally (i.p.), or alternatively were injected daily with 0.5 ml of naïve

Table 1. VSV- and LCMV-neutralizing antibody titers in naïve mice. ND, not determined.

Mice*	Neutralizing titer				
	−βME†		+βME†		−βME†
	VSV-IND	VSV-NJ	VSV-IND	VSV-NJ	LCMV-WE
C57BL/6 (conv.)	1:16	1:16	1:2	1:2	<1:2
C57BL/6 (conv.)	1:16	1:8	<1:2	1:2	<1:2
C57BL/6 (SPF)	1:32	1:16	1:2	1:2	<1:2
C57BL/6 (SPF)	1:16	1:16	1:4	1:2	<1:2
C57BL/6 (GF)	1:32	1:16	1:4	<1:2	ND
C57BL/6 (GF)	1:16	1:16	<1:2	<1:2	ND
KINDG	<1:2	1:16	<1:2	<1:2	ND
KINDG	<1:2	1:16	<1:2	1:2	ND
μMT	<1:2	<1:2	<1:2	<1:2	<1:2
RAG-1$^{-/-}$	<1:2	<1:2	<1:2	<1:2	<1:2

*Sera of conventional (conv.), specific pathogen–free (SPF), and germ-free (GF) C57BL/6 mice were assayed. KINDG, μMT, and RAG-1$^{-/-}$ mice were kept under conventional conditions. Two representative values of 6 to 10 sera tested per group are shown. †Total neutralizing Ig was assessed without incubation with β-mercaptoethanol (−βME); IgG titers represent values after reduction of sera with 0.1 mM β-ME (+βME).

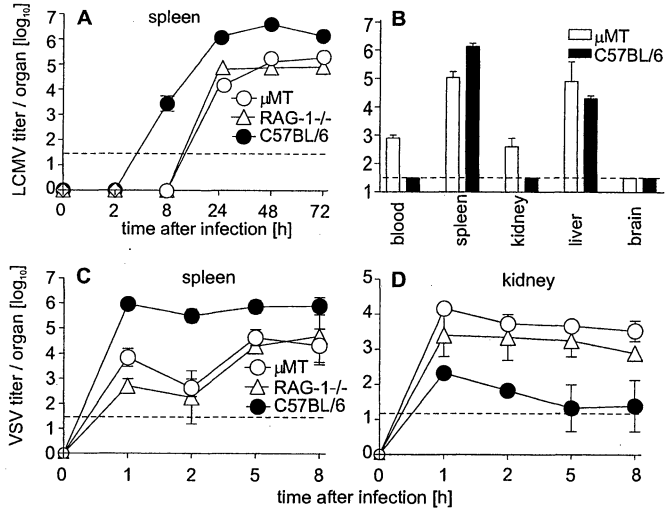

Fig. 2. Viral and bacterial distribution in antibody-deficient and antibody-competent mice. μMT, RAG-1$^{-/-}$, and C57BL/6 (control) mice were infected with 2×10^6 PFU of LCMV-WE i.v., and virus titers in the spleen were determined per organ at the time points indicated (**A**) (*10*). LCMV titers in peripheral organs and in the blood were detectable 24 hours after infection with 2×10^6 PFU and are shown in (**B**). Antibody-deficient mice and C57BL/6 control mice were infected with 2×10^8 PFU of VSV-IND i.v. [subcutaneous injection did not yield detectable virus titers (*9*)]. VSV titers were determined per spleen (**C**) and per kidney (**D**). In (A) through (D), means ± SD of three mice per group are shown, and one of three to five comparable experiments is shown.

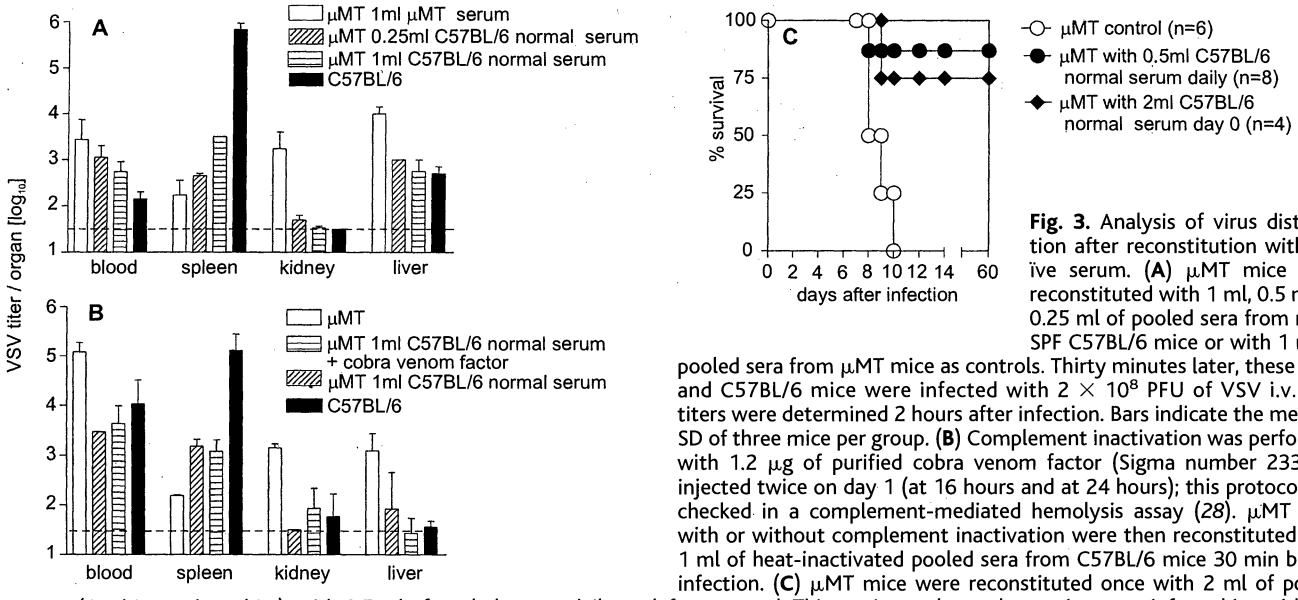

Fig. 3. Analysis of virus distribution after reconstitution with naïve serum. **(A)** μMT mice were reconstituted with 1 ml, 0.5 ml, or 0.25 ml of pooled sera from naïve SPF C57BL/6 mice or with 1 ml of pooled sera from μMT mice as controls. Thirty minutes later, these mice and C57BL/6 mice were infected with 2×10^8 PFU of VSV i.v. VSV titers were determined 2 hours after infection. Bars indicate the mean ± SD of three mice per group. **(B)** Complement inactivation was performed with 1.2 μg of purified cobra venom factor (Sigma number 233550) injected twice on day 1 (at 16 hours and at 24 hours); this protocol was checked in a complement-mediated hemolysis assay (*28*). μMT mice with or without complement inactivation were then reconstituted with 1 ml of heat-inactivated pooled sera from C57BL/6 mice 30 min before infection. **(C)** μMT mice were reconstituted once with 2 ml of pooled naïve serum (1 ml i.v. and 1 ml i.p), with 0.5 ml of pooled serum daily, or left untreated. Thirty minutes later, these mice were infected i.v. with 10^4 PFU of VSV-IND (about 10 times LD_{50}). One of three to four experiments is shown.

serum for 5 days [to compensate for the short in vivo half-life of IgM antibodies of about 20 hours (*19*)] and then challenged with 10^4 PFU of VSV (about 10 times the LD_{50}). All control μMT animals died within 12 days after infection, whereas seven out of eight of those repetitively supplemented and three out of four of the mice given one large dose of normal serum remained healthy during the observation period of 60 days. In addition, the in vivo specificity of natural antibodies was revealed by the heightened susceptibility, by a factor of about 100, of KINDG mice when compared to controls. All KINDG mice died when infected with 10^8 or 10^7 PFU, and one out of three died when infected with 10^6 PFU of VSV, as compared to only one out of three C57BL/6 control mice infected with 10^8 PFU.

Natural antibodies enhance the early recruitment of virus to secondary lymphoid organs after infection with viruses that are known to be controlled by antibodies, such as VSV (*9*), or partially controlled, such as vaccinia virus (*20*), but they also enhance recruitment after infection with pathogens for which antibodies are not thought to be of importance in a primary infection, such as LCMV (*21*) and *Listeria* (*22*). Because no specific Fc receptors for IgM are known, and a direct role of complement was not demonstrable here (Fig. 3B), the formation of virus-antibody complexes, increasing particle size, may be an important factor enhancing phagocytosis and hence uptake from the blood by the spleen. This has been suggested earlier by in vivo studies (*23*) and by in vitro experiments showing that uptake by macrophages is more efficient for large antigen particles or complexes than for small particles (*24*).

A specific B or T cell response is induced in secondary lymphatic organs (*25*). An important protective function of natural antibodies may therefore be not only to influence initial virus distribution and early dissemination to avoid the infection of vital target organs, but also to direct the virus to secondary lymphatic organs in order to accelerate and enhance immune responses. The delayed neutralizing IgG response in $IgM^{-/-}$ mice (*15*) supports this notion. In antibody-deficient μMT mice, the reduction in early filtration of LCMV to the spleen delayed cytolytic T lymphocyte (CTL) activation and allowed the virus to spread to peripheral organs, with the consequence that μMT mice could not control high-dose infections and the CTL response in these mice was exhausted (*26*). In addition, it was shown that μMT mice are resistant to intraperitoneal prion infections (*17*) and that therefore natural or induced antibodies (or B) cells may play a role in prion disease. Collectively, the data show that natural antibodies may well be involved in the recruitment of many pathogens to secondary lymphoid organs and, together with earlier observations (*27*), illustrate an interesting link between innate immunity and the acquired immune responses.

Table 2. Viral and bacterial distribution in antibody-deficient and antibody-competent mice. ND, not determined.

Infection	Comparison of mice*	Number of times that viral or bacterial titer was increased (+) or decreased (−) in antibody-deficient versus antibody-competent mice†				
		Blood	Spleen	Liver	Kidney	Brain
Vacc-WR (5×10^7 PFU)	C57BL/6 versus μMT	+21	−39	+19	+5	ND
Listeria (2×10^7 CFU)	C57BL/6 versus μMT	ND	−79	+3	+3	+13
VSV (2×10^8 PFU)	C57BL/6 versus μMT	+40	−326	+107	+93	+13
	A129 versus AR129	+10	−251	+25	+100	ND
	BALB/c versus $IgM^{-/-}$	+2	−3	+215	+63	ND

*μMT (*14*) mice were on C57BL/6 background; A129 mice (IFN-α/β $receptor^{-/-}$) were compared to AR129 (IFN-α/β $receptor^{-/-}$/RAG-$1^{-/-}$) mice, or $IgM^{-/-}$ mice (*15*) (mice that lack IgM but produce all other immunoglobulin classes) were compared to BALB/c control mice. †Viral organ titers were determined 2 hours after infection with VSV and 6 hours after infection with vacc-WR or *Listeria*. Increase (+) or decrease (−) of viral titers was calculated from mean viral or bacterial organ titers of three to four animals per group. One of two to four comparable experiments is shown.

References and Notes

1. S. Avrameas, *Immunol. Today* **12**, 154 (1991).
2. L. A. Herzenberg and A. B. Kantor, *Immunol. Today* **14**, 79 (1993).
3. G. S. Wilson and A. A. Miles, in *Principles of Bacteriology and Immunity* (Williams and Wilkins, Baltimore, MD, 1961), pp. 1223–1252; R. Gobet *et al.*, *Exp. Cell. Biol.* **56**, 175 (1988).
4. M. C. Carroll, *Annu. Rev. Immunol.* **16**, 545 (1998); B. P. Thornton, V. Vetvicka, G. D. Ross, *J. Immunol.* **152**, 1727 (1994).
5. I. R. Cohen, *Immunol. Rev* **94**, 5 (1986).
6. R. R. Reid *et al.*, *J. Immunol.* **159**, 970 (1997).
7. D. E. Briles *et al.*, *J. Exp. Med.* **153**, 694 (1981).
8. A. Coutinho, *Immunol. Rev.* **110**, 63 (1989).
9. R. R. Wagner, *The Rhabdoviruses* (Plenum, New York, 1987); B. Dietzschold *et al.*, in *Virology*, B. N. Fields, D. M. Knipe, A. M. Howley, Eds. (Raven, New York, 1995), pp. 1137–1159.

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.
13. M. F. Bachmann *et al*, *Science* **262**, 1448 (1993).
14. D. Kitamura, J. Roes, R. Kühn, K. Rajewski, *Nature* **350**, 423 (1991); P. Mombaerts *et al.*, *Cell* **68**, 869 (1992).
15. C. Lutz *et al.*, *Nature* **393**, 797 (1998).
16. U. Müller *et al*,. *Science* **264**, 1918 (1994); U. Steinhoff *et al.*, *J. Virol.* **69**, 2153 (1995).
17. M. A. Klein *et al.*, *Nature* **390**, 687 (1997); R. Frigg, M. A. Klein, R. M. Zinkernagel, A. Aguzzi, *J. Virol.* **73**, 9584 (1999).
18. A. R. Thomsen *et al.*, *Int. Immunol.* **9**, 1757 (1997). Thomsen *et al.* reconstituted μMT mice with sera containing VSV-specific IgM or IgG antibodies and with sera of unimmunized mice as a control. After challenge with 10^6 PFU of VSV, only sera containing VSV induced specific antibodies but not normal serum-mediated protection. The dose used in these experiments was about 100 to 1000 times above LD_{50} for μMT mice.
19. P. Vieira and K. Rajewsky, *Eur. J. Immunol.* **18**, 313 (1988).
20. F. Fenner, B. R. McAuslan, C. A. Mims, J. Sambrook, D. O. White, *The Biology of Animal Viruses* (Academic Press, London, 1974).
21. M. J. Buchmeier, R. M. Welsh, F. J. Dutko, M. B. A. Oldstone, *Adv. Immunol.* **30**, 275 (1980); A. R. Thomsen, J. Johansen, O. Marker, J. P. Christensen, *J. Immunol.* **157**, 3074 (1996).
22. J. Rothe *et al.*, *Nature* **364**, 798 (1993); S. H. E. Kaufmann, *Annu. Rev. Immunol.* **11**, 129 (1993).
23. P. C. Frei, S. Cruchaud, L. Arnaud, A. Vannotti, *J. Immunol.* **101**, 605 (1968); W. O. Weigle, *Adv. Immunol.* **16**, 61 (1973).
24. M. C. Lamers, E. R. De Groot, D. Roos, *Eur. J. Immunol.* **11**, 764 (1981).
25. U. Karrer *et al.*, *J. Exp. Med.* **185**, 2157 (1997); A. F. Ochsenbein *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 2233 (1999).
26. M.-A. Bründler *et al.*, *Eur. J. Immunol.* **26**, 2257 (1996).
27. D. T. Fearon and R. M. Locksley, *Science* **272**, 50 (1996); M. R. Ehrenstein, T. L. O'Keefe, S. L. Davies, M. S. Neuberger, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 10089 (1998).
28. T. Fehr *et al.*, *Cell. Immunol.* **168**, 184 (1996).
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

David A. Randolph,[1,2] Guangming Huang,[3] Cynthia J. L. Carruthers,[3] Lindsay E. Bromley,[3] David D. Chaplin[1,2,3]*

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

[1]Division of Allergy and Immunology, Department of Internal Medicine; [2]Center for Immunology, Washington University School of Medicine; [3]Howard Hughes Medical Institute, St. Louis, MO 63110, USA.

*To whom correspondence should be addressed at Howard Hughes Medical Institute, Washington University School of Medicine, 660 South Euclid Avenue, Box 8022, St. Louis, MO 63110, USA. E-mail: chaplin@im.wustl.edu

References and Notes

[13] **The Influence of Antigen Organization on B Cell Responsiveness**

Martin F. Bachmann; Urs Hoffmann Rohrer; Thomas M. Kündig; Kurt Bürki; Hans Hengartner; Rolf M. Zinkernagel

Science, New Series, Vol. 262, No. 5138. (Nov. 26, 1993), pp. 1448-1451.

Stable URL:

http://links.jstor.org/sici?sici=0036-8075%2819931126%293%3A262%3A5138%3C1448%3ATIOAOO%3E2.0.CO%3B2-G

[16] **Functional Role of Type I and Type II Interferons in Antiviral Defense**

Ulrike Müller; Ulrich Steinhoff; Luiz F. L. Reis; Silvio Hemmi; Jovan Pavlovic; Rolf M. Zinkernagel; Michel Aguet

Science, New Series, Vol. 264, No. 5167. (Jun. 24, 1994), pp. 1918-1921.

Stable URL:

http://links.jstor.org/sici?sici=0036-8075%2819940624%293%3A264%3A5167%3C1918%3AFROTIA%3E2.0.CO%3B2-W

[25] **Immune Surveillance Against a Solid Tumor Fails because of Immunological Ignorance**

Adrian F. Ochsenbein; Paul Klenerman; Urs Karrer; Burkhard Ludewig; Marcus Pericin; Hans Hengartner; Rolf M. Zinkernagel

Proceedings of the National Academy of Sciences of the United States of America, Vol. 96, No. 5. (Mar. 2, 1999), pp. 2233-2238.

Stable URL:

http://links.jstor.org/sici?sici=0027-8424%2819990302%2996%3A5%3C2233%3AISAAST%3E2.0.CO%3B2-F

LINKED CITATIONS

[27] **The Instructive Role of Innate Immunity in the Acquired Immune Response**
Douglas T. Fearon; Richard M. Locksley
Science, New Series, Vol. 272, No. 5258. (Apr. 5, 1996), pp. 50-54.
Stable URL:
http://links.jstor.org/sici?sici=0036-8075%2819960405%293%3A272%3A5258%3C50%3ATIROII%3E2.0.CO%3B2-3

[27] **Targeted Gene Disruption Reveals a Role for Natural Secretory IgM in the Maturation of the Primary Immune Response**
Michael R. Ehrenstein; Theresa L. O'Keefe; Sarah L. Davies; Michael S. Neuberger
Proceedings of the National Academy of Sciences of the United States of America, Vol. 95, No. 17. (Aug. 18, 1998), pp. 10089-10093.
Stable URL:
http://links.jstor.org/sici?sici=0027-8424%2819980818%2995%3A17%3C10089%3ATGDRAR%3E2.0.CO%3B2-U