The Role of B-1 and B-2 Cells in Immune Protection from Influenza Virus Infection

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Introduction

Two distinct B cell populations, B-1 and B-2, which are present in the periphery of adult man and mice, differ considerably in development, surface phenotype, immunoglobulin-repertoire usage, and tissue distribution [1, 2]. Importantly, these types of B cells also differ in their responses to stimulation with antigen. Conventional B cells (B-2), which constitute most of the B cells in secondary lymphoid tissues of adults, undergo rapid proliferation when activated with anti-IgM antibody Fab_2 fragments. In contrast, ($CD5^+$) B-1 cells, which constitute the main peripheral B cell population in neonates and the main B cell population in the coelomic cavities of adults, undergo apoptosis rather then proliferation when stimulated through their B cell receptor [3-5]. On the other hand, B-1 cells, like their B-2 counterparts proliferate vigorously when stimulated with mitogens such as lipopolysaccharides [3, 5, 6], indicating that antigen recognition by B-1 and B-2 cells *in vivo* can, depending on the type of antigen encountered, induce different response patterns.

What the exact function B-1 cells is during immune responses to pathogens is still unresolved. B-1 cells are the producers of most of the circulating "natural" antibodies [7-9] and produce antibodies to a number of evolutionary conserved bacterial cell wall antigens, such as phosphoryl choline and phosphatidyl choline (PtC) (Reviewed in [10]). Recently, it was demonstrated that B-1 cell-derived natural IgM antibodies specific for PtC protect mice from rapid death following cecal ligation- and puncture-induced bacterial sepsis [11]. Thus, the study supports the long held view that B-1 cell-derived IgM antibodies constitute a first-line of defense against infection with bacteria [12]. The role of B-1 cells in immune protection from viral infections has not been studied. *In vitro* studies by Rott and colleagues [13] had shown, however, that certain strains of influenza virus act as mitogens for B-1 cells, inducing T-independent proliferation of these cells. We therefore compared the *in vivo* responses of B-1 and B-2 cells to infection with influenza virus and delineated their contribution to the protective immunity against this virus.

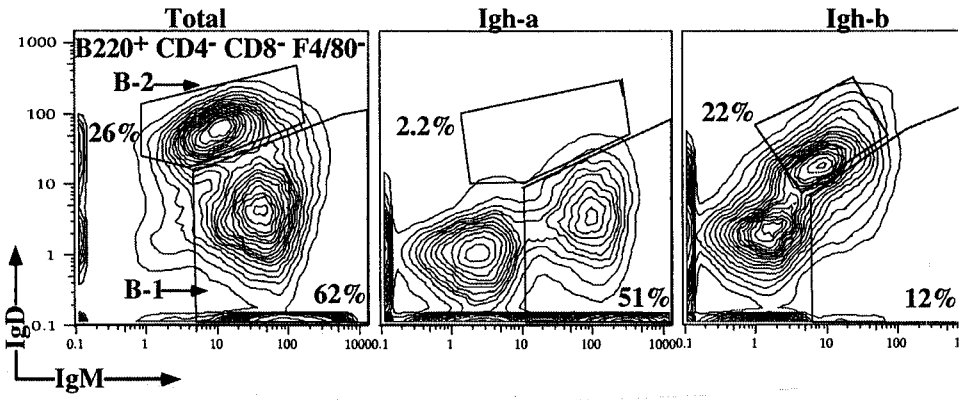

Fig. 1. Peritoneal cavity of B-1/B-2 allotype chimeras contain B-1 and B-2 cells of differing allotypes. Shown are 5% contour FACS plots of PerC from anti-IgM^b antibody-treated (Igh-b) CB.17 mice reconstituted with PerC from (Igh-a) BALB/c mice two months after end of antibody treatment. Cells were gated for expression of the B cell marker B220 and lack of expression of T cell and macrophage markers as indicated, and then stained with either total or allotype-specific anti-IgM and anti-IgD. Frequencies of B-1 and B-2 cells are indicated.

The Innate and Acquired Humoral Response to Influenza Virus is Provided by B-1 and B-2 Cells, Respectively

We created B-1/B-2 allotype chimeric mice in order to distinguish the cells and cell products *in vivo* using allotype-specific monoclonal antibodies against various classes of Ig [14]. Newborn Igh-b allotype-expressing CB.17 mice are treated from birth with anti-IgM^b antibodies to ablate host-derived B cell development. On day two after birth these mice receive peritoneal cavity wash out cells (PerC) as a source of B-1 cells, or FACS-sorted B-1 cells, from Igh-a allotype-expressing congenic BALB/c mice. The antibody treatment is continued for 6 weeks during which donor-derived B-1 cells expand. Within two months after the end of antibody treatment, host-derived B cell development leads to full reconstitution of the B-2 cell compartment. For at least 16 month after cell transfer, however, more then 80% of the B-1 cell compartment in peritoneal cavity and spleen of these chimeras consist of donor-derived B-1 cells. A maximum of 20% of the B-1 cell pool is derived from the host (Fig. 1). Thus, all Igh-a expressing B cells in these mice are B-1 cells and the vast majority of the Igh-b expressing B cells are B-2 cells. Sublethal infection of the chimeras was induced with the influenza A virus strain "Mem71" [15].

The results were clear-cut [8]. Whereas B-1 cells produce most (>80%) of the circulating total IgM, including the virus-binding natural IgM antibodies that are present prior to the infection, B-2 cells contribute only minimally, if at all, to the

Table 1. Total and virus-specific serum IgM and IgG2a levels in allotype-chimeras and controls

Mouse strain	Days after infection	Ig-allotype	Total IgM (μg/ml)*	Anti-Mem71 IgM (U/ml)*	Anti-Mem71 IgG2a (U/ml)*
Chimera	0	Igh-a	2,900	8	<0.001
	7	Igh-a	n.d.	9	<0.001
	0	Igh-b	400	3	<0.001
	7	Igh-b	n.d.	50	8,000
BALB/c	0	Igh-a	2,600	8	<0.001
	7	Igh-a	n.d	160	1,000
CB.17	0	Igh-b	2,900	20	<0.001
	7	Igh-b	n.d.	80	20,000

n.d., not determined; *mean levels

serum-IgM pool and make little natural anti-viral IgM (Table 1). In contrast, seven days after influenza virus infection the observed increases in the titers of virus-specific serum IgM were of the b-allotype, whereas the levels of Igh-a allotype virus-specific IgM were unaltered (Table 1). Similar results were obtained with a kinetic study in which antibody titers were measured daily for two weeks after the infection [8]. Thus, B-1 cells do not respond to the infection with increased IgM production. All increased IgM production is derived from B-2 cells.

In the chimeras, roughly similar amounts of total serum IgG2a is produced by B-1 and B-2 cells. Neither B-1 nor B-2 cell-derived virus-binding antibodies of the IgG2a isotype were detected prior to influenza virus infection, but they make the bulk of the virus-specific humoral response following infection [16, 17]. Similar to the IgM response, all of the virus-induced specific IgG2a in the chimera was secreted by the B-2 cells. Therefore, the natural IgM secreting B cells are not the precursors of the cells that respond to the infection with further IgM secretion, or the precursor cells that start the germinal center reaction for affinity maturation and isotype switching. B-1 cells function by providing steady-state levels of natural virus-binding IgM antibodies, unaffected by the presence of antigen. In contrast, B-2 cells function by rapidly inducing the secretion of virus-specific IgM and IgG antibodies, which are presumably of higher affinity then those secreted by the B-1 cells. Maintaining normal levels of poly-specific natural antibodies throughout an infection may be of importance for the maintenance of immune protection against infections with other pathogens, at a time when the adoptive immune system is engaged in the response to the primary pathogen. B-1 cell-derived IgM might therefore serve a function distinct from that of B-2 cell-derived IgM.

Non-Redundant Role for B-1 and B-2 Cells in influenza Virus Infection

We wanted to test the role of secreted IgM for immune protection from influenza virus infection. For this, we compared rates of survival in wild-type mice and mice deficient in the secreted, but not membrane-bound form of IgM ($sIgM^{-/-}$) [18]

following infection with influenza virus. Indeed, despite the fact that sIgM$^{-/-}$ mice have otherwise normal populations of B cells able to secrete all other classes of immunoglobulins, the lack of secreted IgM alone led to a significant increase in deaths from infection [19]. Roughly 50% of sIgM$^{-/-}$ succumbed to infection with a dose of influenza virus that does not cause deaths in the wild-type controls. These increased deaths in the sIgM$^{-/-}$ mice were associated with a significant increase in lung virus titers at days 5 and 7, and a significant reduction in the virus-specific serum IgG response during the first 3 weeks following infection, when compared to controls (Fig.2).

To delineate the contributions of the B-1 and B-2 cell-derived IgM antibodies for this strong immune protective effect, we created B-1/B-2 irradiation chimeras between sIgM$^{-/-}$ mice and wild-type controls. Groups of irradiated sIgM$^{-/-}$ mice were reconstituted with sIgM$^{-/-}$ PerC and wild-type bone marrow and vise versa to create mice that lack either secreted IgM from the B-1 cells or from the B-2 cells. Surprisingly, following influenza virus infection, both groups of chimeras had mortality rates similar to mice in which neither B-1 nor B-2 cells secreted IgM. Consistent with the increased deaths seen in both groups of chimeras, these mice had also significant lower IgG2a responses compared to controls that had received PerC and bone marrow from wild-type mice. Furthermore, reconstitution of sIgM$^{-/-}$ mice with purified serum IgM from normal mice did not alter the mortality rates, or the levels of virus-specific IgG2a. Hence, secretion of IgM by both B-1 and B-2 cells is necessary for maximal immune protection against an acute viral infection. The presence of only one type of IgM is insufficient to provide full immune protection.

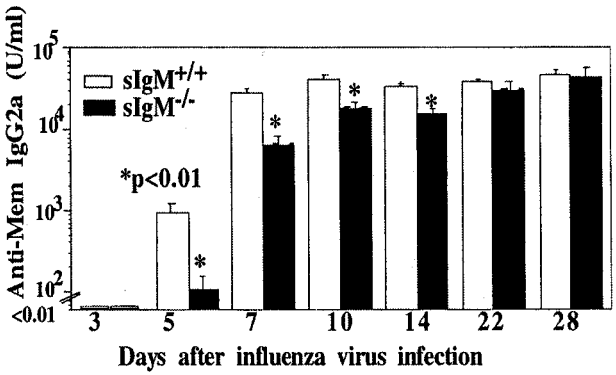

Fig. 2. Reduced anti-viral serum IgG2a titers in mice lacking secreted IgM. sIgM$^{-/-}$ mice and wild type controls (sIgM$^{+/+}$) were infected with influenza virus Mem71 and serum levels of virus-specific IgG2a were determined by ELISA. Arbitrary units were determined by comparison to an allotype matched hyperimmune serum.

How does secreted IgM provide immune protection against a viral infection? Various effects might operate simultaneously. IgM antibodies can neutralize the virus directly to prevent virus-attachment and internalization by host epithelial cells [20]. Moreover, our data show that secreted IgM affects virus neutralization in part indirectly by regulating the magnitude of the virus-specific (neutralizing) IgG response. Other effects such as opsonization of influenza virus for increased uptake by alveolar macrophages through binding of antigen-IgM-complement complexes to complement receptors might also play a role.

Secreted IgM Functions as Autocrine Regulator of the IgG Response

The requirement for secreted IgM in induction of optimal anti-viral IgG responses demonstrated here for influenza virus, is consistent with previous observations showing that secreted IgM is required for efficient IgG antibody responses to suboptimal doses of T-dependent antigens [18, 21]. This raises the question of how secreted IgM regulates the IgG response. Since complement and complement receptors are crucial for the induction of normal antibody responses [22, 23], previous studies using $sIgM^{-/-}$ mice proposed that natural IgM antibodies augment IgG responses by activating complement to form immune complexes [18, 21]. These complexes may then activate B cells by cross-linking the B cell receptors and/or by being trapped on follicular dendritic cells for activation of efficient germinal center reactions. Virus-induced B-2 cell-derived IgM is more effective in promoting the IgG response after influenza virus infection than B-1 cell-derived natural IgM. Chimeras that lacked the B-2 cell-derived IgM showed a more dramatic reduction in virus-specific IgG2a titers compared to chimeras that lacked B-1 cell-derived IgM [19]. Thus, focal secretion of virus-specific IgM immediately following B-2 cell activation seems crucial for directly activating the IgM-secreting B cells for IgG production.

As both virus-induced IgM and IgG are derived from B-2 cells, secreted IgM seems to act in an autocrine fashion. Since IgM-antigen complexes activate the classical complement cascade, antigen-IgM complexes present in the vicinity of the secreting antigen-specific B cells may bind to complement receptors expressed by these cells. These receptors are known to provide important costimulatory signals [22, 23], thus complement receptor engagement may further stimulate the antigen-activated IgM-secreting B cells. In addition, polymeric IgM may enhance antigen-mediated B cell receptor triggering by cross-linking surface Ig receptors on B cells that have bound the antigen.

T-independent secretion of IgM is induced in response to a number of antigens, including vesicular stomatitis virus [24]. It is therefore possible that the early activation of B cells in response to a viral infection is regulated independently of a helper T cell response. The early secretion of IgM by antigen-specific B cells might induce or enhance initial clonal expansion of these B cells in a manner

similar to that of the early autocrine secretion of IL-2 by antigen-stimulated T cells which enhances clonal expansion of the T cells. Enhanced precursor frequencies of antigen-specific B cells increase the chances of T-B interaction, and therefore the speed and magnitude of T cell-dependent B cell differentiation events such as isotype switching and affinity maturation. Although speculative at this point, if these events are proven to occur *in vivo*, such an initial T cell-independent activation of B cells would bring into question the validity of the current two-signal model of T cell-dependent B cell activation.

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References

1. Stall AM, Wells SM, Lam K-P (1996) B-1 cells: unique origins and functions. Seminars in Immunology 8:45 - 59
2. Kantor AB, Herzenberg LA (1993) Origin of murine B cell lineages. Annu. Rev. Immunol 11:501 - 538
3. Rothstein TL, Kolber DL (1988) Anti-Ig antibody inhibits the phorbol ester-induced stimulation of peritoneal B cells. J. Immunol. 141:4089 - 4093
4. Nawata Y, Stall AM, Herzenberg LA, Eugui EM, Allison AC (1990) Surface immunoglobulin ligands and cytokines differentially affect proliferation and antibody production by human CD5+ and CD5- B lymphocytes. Int. Immunol. 2:603 - 614
5. Bikah G, Carey J, Ciallella JR, Tarakhovsky A, Bondada S (1996) CD5-mediated negative regulation of antigen receptor-induced growth signals in B-1 B cells. Science 274:1906 - 1909
6. Morris DL, Rothstein TL (1993) Abnormal transcription factor induction through the surface immunoglobulin M receptor of B-1 lymphocytes. J. Exp. Med 177:857 - 861
7. Avrameas S (1991) Natural autoantibodies: from "horror autotoxicus" to "gnothi seauton" Immunol. Today
8. Baumgarth N, Herman OC, Jager GC, Herzenberg LA, Herzenberg LA (1999) Innate and acquired Immunities to influenza virus are provided by distinct B cells. Proc. Natl. Acad. Sci USA 96:2250 - 2255
9. Coutinho A, Kazatchkine MD, Avrameas S (1995) Natural autoantibodies. Curr. Opin Immunol. 7:812 - 818
10. Allison AC, Nawata Y (1992) Cytokines mediating the proliferation and differentiation of B-lymphocytes and their role in ontogeny and phylogeny. Annn. N. Y. Acad. Sci 651:200 - 219
11. Boes M, Prodeus AP, Schmidt T, Carroll MC, Chen J (1998) A critical role of natural immunoglobulin M in immediate defense against systemical bacterial infection. J. Exp. Med 188:2381 - 2386
12. Herzenberg LA, Herzenberg LA (1989) Towards a layered immune system. Cell 59:953 - 954
13. Cash E, Charreire J, Rott O (1996) B-cell activation by superstimulatory influenza virus hemagglutinin: a pathogenesis for autoimmunity? Immunol. Rev. 152:67 - 88
14. Lalor PA, Stall AM, Adams S, Herzenberg LA (1989) Permanent alteration of the murine Ly-1 B repertoire due to selective depletion of Ly-1 B cells in neonatal animals. Eur. J. Immunol 19:501 - 506

15. Baumgarth N, Brown L, Jackson D, Kelso A (1994) Novel features of the respiratory tract T-cell response to influenza virus infection: lung T cells increase expression of gamma interferon mRNA in vivo and maintain high levels of mRNA expression for interleukin-5 (IL-5) and IL-10. J. Virol. 68:7575 - 7581
16. Hocart MJ, Mackenzie MJS, Steward GA (1988) The IgG subclass responses induced by wild-type, cold-adapted and purified haemagglutinin from influenza virus A/Queensland/6/72 in CBA/CaH mice. J. General Virol. 69:1873 - 1882
17. Coutelier J-P, Logt JTMVd, Heessen FWA, Warnier G, Snick JV (1987) IgG2a restriction of murine antibodies elicited by viral infections. J. Exp. Med. 165:64 - 69
18. Boes M, Esau C, Fischer MB, Schmidt T, Carroll M, Chen J (1998) Enhanced B-1 cell development, but impaired IgG antibody responses in mice deficient in secreted igM. J. Immunol 160:4776 - 4787
19. Baumgarth N, Herman OC, Jager GC, Brown L, Boes M, Herzenberg LA, Chen J (1999) B-1 and B-2 cell-derived IgM antibodies are non-redundant components of the protective response to influenza virus infection, *submitted for publication.*
20. Taylor HP, Dimmock NJ (1985) Mechanisms of neutralization of influenza virus by IgM. J. Gen. Virol. 66:903 - 907
21. Ehrenstein MR, O'Keefe TL, Davies SL, Neuberger MS (1998) Targeted gene disruption reveals a role for natural secretory IgM in the maturation of the primary immune response. Proc. Natl. Acad. Sci USA 95:10089 - 1093
22. Tedder TF, Inaoki M, Sato S (1997) The CD19- CD21 complex regulates signal transduction thresholds governing humoral immunity and autoimmunity. Immunity 6:107 - 118
23. Carroll MC (1998) The role of complement and complement receptors in induction and regulation of immunity. Annu. Rev. Immunol. 16:545 - 568
24. Bachmann MF, Zinkernagel RM (1997) Neutralizing antiviral B cell responses. Annu. Rev. Immunol. 15:235 - 270