Correlation of T Cell Independence of Antibody Responses with Antigen Dose Reaching Secondary Lymphoid Organs: Implications for Splenectomized Patients and Vaccine Design

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Many natural viral and bacterial pathogens activate B cells independently of Th cells (TI Ags). This study analyzed the characteristics of the activation of B cells after immunization with various forms of viral Ags using different immunization routes and found a decreasing dependence on T help with increasing amounts of Ag recruited to the spleen. Repetitive antigenic structure facilitated TI B cell responses if Ag was present in lymphoid organs. These results suggest that 1) Ag dose and localization in secondary lymphoid organs are the key for B cell activation in the absence of T help; 2) early TI Ab responses are crucial to protect against systemically spreading acute cytopathic infectious agents; and 3) there may be new rationales for improved vaccine design. The Journal of Immunology, 2000, 164: 6296–6302.

Infectious pathogens usually enter the organism via mucosal surfaces and are controlled by a locally induced immune response (mainly IgA) (1, 2). A key step in the pathogenesis of an infectious disease is the hematogenic spread of the virus or bacterium to reach target organs that may be vital (1). The immunological control of a viremia or a bacteremia is therefore important to prevent a potentially lethal disease; this goal is achieved by most of the available efficient vaccines and correlates largely with neutralizing Ab titers.

Three modes of Ab production, natural or spontaneous, T independent (TI), and T dependent (TD), potentially play roles in the protection against hematogenically spreading pathogens. Natural Abs representing the natural B cell repertoire are spontaneously, constitutively secreted without specific stimulation mainly by peritoneal B-1 cells (3, 4) and therefore represent a first line of defense against various pathogens (5). Specific B cells are activated in two different ways in vivo. Repetitively arranged Ags, such as haptenated synthetic polymers and neutralizing determinants of viruses (6), or polyclonal B cell activators, such as LPS, do not need second signals provided by cognate helper cells to induce B cell responses (6–9). TI B cell activation has been correlated with antigenic structure (6, 10). For example, low density haptenated beads act as TD Ags, whereas haptenated beads at high density acted as TI Ags. Using linear polymers or viruses, the two-dimensional spacing of 5–10 nm has been shown to be optimal for TI induction of B cells (6, 11, 12). Bacteria or viruses often directly elicit strong TI Ab responses (13).

B cell activation by T cell-dependent Ags and generation of B cell memory requires two signals; the first signal is mediated by the occupied B cell receptor, and the second signal is usually provided by Th cells (14, 15). The actual priming of naïve T cells is mediated by dendritic cells and probably by macrophages (16, 17). For the generation of TD humorol immune responses against proteins, adjuvants and booster immunizations are needed to obtain high Ab titers (18).

During hematogenic spread of an infection the host has to eliminate large quantities of Ag quickly to prevent the spread of the pathogen to peripheral vital organs. As a consequence, high titers of neutralizing Abs have to be elicited rapidly before or at the time the pathogen is reaching systemic circulation. Because induction of T help requires 4–6 days (19–21), early TI Ab responses might be crucial for survival. Therefore, the present study evaluated the role and induction requirements of TI and TD Ab responses in controlling viremia and in host protection.

Materials and Methods

Mice

C57BL/6 and BALB/c mice were purchased from the Institute for Laboratory Animals (Veterinary Hospital, Zurich, Switzerland). CBA/xid and CBA/N controls were purchased from Harlan (Bicester, U.K.). Experiments were performed in a conventional mouse house facility, and mice were used at 6–12 wk of age.

Viruses and viral Ags

Vesicular stomatitis virus (VSV) Indiana (VSV-IND; Mudd-Summers isolate) and VSV New Jersey (VSV-NJ; Pringle isolate) were originally obtained from Dr. D. Kolakovsky (University of Geneva, Geneva, Switzerland) and were grown on BHK21 cells. Lympohocytic choriomeningitis virus (LCMV)-WE were originally obtained from Dr. F. Lehmann Grube (Heinrich Pette Institute, Hamburg, Germany) and was propagated on L929 fibroblast cells. Poliovirus stock solutions of serotype II were obtained from the Swiss Serum and Vaccine Institute (Bern, Switzerland). Inactivated poliovirus vaccine containing all three major serotypes (Salk, formaldehyde fixed) was purchased from BERNIA (Bern, Switzerland). Recombinant baculoviruses expressing the glycoprotein of VSV (VSV G) and the nucleoprotein of LCMV (LCMV-NP) were gifts from Dr. D. H. L. Bishop,
FIGURE 1. Th cell dependence of Ab responses after immunization with different Ag doses and i.v. and s.c. immunization routes. C57BL/6 mice were either depleted of CD4+ T cells by injection of anti-CD4 Abs 3 days and 1 day before infection or were left untreated. A, Mice were then infected with 5 × 10^3 or 200 PFU of LCMV i.v., and LCMV-NP-specific Abs were assessed by ELISA at the time points indicated. B, CD4+ T cell-depleted and untreated control mice were infected with 10^6 PFU of VSV i.v., 200 PFU of VSV i.v., or 5 × 10^3 PFU of VSV s.c., and neutralizing IgM Ab titers were measured at the time points indicated. C, Similarly, CD4+ T cell-depleted and control C57BL/6 mice were infected with 10^5 PFU of VSV i.v., 10^5 PFU of VSV G i.v., or with 5 × 10^5 PFU of VacV VSV G s.c. D, VSV G protein was injected i.v. (20 or 1 μg), or 20 μg of VSV G protein in IFA was injected s.c. into the flank of CD4+ T cell-depleted or control mice. E, BALB/c mice were either depleted of CD4+ T cells or left untreated and infected with 500 μl i.v., 25 μl i.v., or 500 μl s.c. of poliomyelitis virus in IFA. Neutralizing IgG (distinguished from IgM by reduction with 0.1 M 2-ME; data not shown) on day 8 after immunization was at least two titer steps lower than total Ig, indicating that the Abs measured until day 8 largely represented neutralizing IgM. All experiments were repeated twice in CD4+ T cell-depleted mice and in nude mice with comparable results. Results are given as the mean ± SD of three or four mice per group.

NERC Institute of Virology (Oxford, U.K.). They were derived from nuclear polyhedrosis virus and were grown at 28°C in Spodoptera frugiperda cells in Spinner cultures (22).

VSV and poliomyelitis virus neutralization assay

Serum from immunized mice was prediluted 40-fold in MEM containing 2% FCS. Serial 2-fold dilutions were mixed with equal volumes of VSV (500 PFU/ml) and incubated for 90 min at 37°C in an atmosphere with 5% CO2. One hundred microtiter of the serum-virus mixture was transferred onto Vero cell monolayers in 96-well plates and incubated for 1 h at 37°C. The monolayers were overlaid with 100 μl of DMEM containing 1% methylcellulose and incubated for 24 h at 37°C. The overlay was flicked off, and the monolayer was fixed and stained with 0.5% crystal violet. The highest dilution of serum that reduced the number of plaques by 50% was taken as the titer. To determine IgG titers, undiluted serum was pretreated with an equal volume of 0.1 M 2-ME in saline. Poliomyelitis virus neutralization assays were performed similarly, but samples were prediluted 1/20 (23).

LCMV-NP-specific ELISA

We used an ELISA with the following steps: 1) coating with baculovirus-derived LCMV-NP (1 μg/ml); 2) blocking with 2% BSA (Fluka, Buchs, Switzerland) in PBS; 3) addition of 10-fold prediluted sera, titrated 1/3 over 12 dilution steps; 4) detection with IgM- or IgG-specific HRP-labeled goat anti-mouse Abs (0.5 μg/ml; Southern Biotechnology Associates, Birmingham, AL); and 5) addition of substrate 2,2′-azino-bis-[3-ethylbenzthiazoline-6-sulfonate] (Roche, Mannheim, Germany) and H2O2 (Fluka). Plates were coated overnight at 4°C, all other incubations were conducted for 60–90 min at room temperature. Between incubations, plates were washed three times with PBS containing 0.05% Tween 20. OD was measured at 405 nm in an ELISA reader, and Ab titers were determined as the serum dilutions yielding an absorption of twice background levels. In vivo CD4+ T cell depletion

Mice were treated i.p. on days −3 and −1 before infection with 1 mg of anti-CD4 mAb YTS191.6 (24). This treatment completely abrogates the switch from IgM to IgG and depletes CD4+ Th cells to below the detection level by FACS analysis (not shown).

Immunohistochemistry

Freshly removed organs were immersed in HBSS and snap-frozen in liquid nitrogen. Tissue sections 5 μm thick were cut in a cryostat, placed on siliconized glass slides, air-dried, fixed with acetone for 10 min, and stored at −70°C. Staining for VSV Ag or VSV-specific B cells was performed as previously described (25).

Splenectomy and intrasplenic/intra-lymph node injections

After ether anesthesia was administered to the mice, spleens were prepared for splenectomy, and the afferent and the efferent blood vessels were coagulated with a heated needle. Similarly, intrasplenic injections or injections directly into a lymph node were given in ether anesthesia after preparation of the spleen or mesenteric lymph nodes. The peritoneal cavity was closed with a silk suture, and the skin was stapled.

Results

Role of Ag dose and immunization route

Mice were infected with different doses of replicating or nonreplicating virus or with recombinant viral protein to analyze the Th cell (CD4+ T cell) dependence of the resulting Ab response. Experiments were performed in C57BL/6 and BALB/c mice depleted of CD4+ T cells by repetitive injection of CD4+ T cell-depleting Abs (24) (Fig. 1) or in T cell-deficient nude mice (not shown, but...
yielding comparable results). This protocol resulted in CD4+ T cell levels below the detection limit by FACS analysis, and the efficiency of depletion was confirmed by a complete block of the switch to IgG in treated animals (not shown). The level of neutralizing IgG (distinguished from IgM by reduction with 0.1 M 2-ME, an unequivocal means of destroying IgM (26)) on days 6 and 8 after immunization was at least 4-fold (= 2 log₂ steps) lower than that of total Ig (data not shown), indicating that the Abs measured until day 8 largely represented neutralizing IgM.

First, dose responses of systemic i.v. infections or immunizations were assessed. High dose infection (Fig. 1, top row) with the noncytopathic arenavirus LCMV (Fig. 1A), the highly cytopathic rhadovirus VSV (Fig. 1B), recombinant vaccinia virus expressing the glycoprotein of VSV (Vacc VSV G; Fig. 1C), or poliomyelitis virus (Fig. 1E) elicited IgM Ab responses that were largely independent of CD4+ Th cells (anti-NP of LCMV Abs was assessed by ELISA; Vacc VSV G-, or poliomyelitis virus-specific Abs were tested in neutralization assays). In contrast, a low dose infection (Fig. 1, middle row) with these viruses led to a largely T-dependent Ab response. These results were confirmed in mice immunized with recombinant baculovirus-derived VSV proteins (Fig. 1D, VSV G protein) in which i.v. injection of 20 μg (Fig. 1D, top panel) or 1 μg (Fig. 1D, middle panel) lead to a TI or TD Ab response, respectively.

Most viruses and bacteria infect hosts via peripheral routes, often via mucosal surfaces and not directly i.v. (with the exception of arthropode-borne viruses (1), septicaemias, or pyemias). Therefore, an s.c. route of infection or immunization was evaluated for the induction of TI Ab responses. Interestingly, Ab responses after s.c. injection of a high infectious dose of VSV and poliomyelitis virus or a high dose of a VSV model Ag were dependent on the presence of CD4+ T cells (Fig. 1, bottom row). Surprisingly, high doses of poliomyelitis virus failed to directly elicit an Ab response after s.c. injections (not shown); however, after s.c. injection in IFA inducing and providing efficient T help (14), neutralizing Ab responses became detectable.

VSV and poliovirus exhibit their neutralizing determinants in a repetitive form (1, 11). In contrast, VSV protein or LCMV-NP released from infected cells forms micelles with a low degree of organization (27), and Vacc VSV G proteins are only expressed in the lipid bilayer membrane of infected cells without any strict organization (28). The results presented so far suggest that although the structure of the Ag plays a role (6, 12, 13) (see below), the type of virus was key in determining whether one and the same Ag was a strong TI-1 Ag, a weak TI-2 Ag, or even strictly dependent on cognate T help.

Role of secondary lymphoid organs

The finding that not only the infectious dose by itself but also the route of Ag administration determine the mode of the B cell activation suggested that dependence on T help is determined by the amount of Ag reaching secondary lymphoid organs. To reduce efficient Ag trapping to the spleen after systemic infection or immunization i.v., mice were splenectomized. Interestingly, i.v. infection of splenectomized C57BL/6 mice with live VSV (Fig. 3, A1 and A2) or with Vacc VSV G (Fig. 3, A3 and A4) led to a delayed and reduced IgM response that, unexpectedly and in contrast to that in unmanipulated control mice, was largely dependent on T help, as documented by the susceptibility of Ab responses to CD4+ T cell depletion and the delay of the Ab response in splenectomized, compared with control, mice (Fig. 3, A1 vs A2 and A3 vs A4, respectively). The residual Ab titer induced in CD4+ T cell-depleted splenectomized mice may be a consequence of T help provided by γδ T cells (33) or may reflect some trapping of Ag in lymph nodes.

In contrast to replicating virus, inactivated or attenuated viruses have been shown to induce only very limited T help (34). A corresponding shift in overall Ab responses was revealed in splenectomized C57BL/6 mice immunized with nonreplicating UV-inactivated VSV (Fig. 3B) or with inactivated poliomyelitis virus (Fig. 3C). The nonreplicating Ag failed to elicit Ab responses in splenectomized mice (Fig. 3, B and C). These results are reminiscent of earlier experiments that have shown that spleenectomy completely blocks the Ab response to certain nonreplicating Ags (35, 36). The present results further support the view that live replicating pathogens provide IL environments that enhance T help (34).
FIGURE 3. Induction of anti-viral Ab responses in splenectomized mice. C57BL/6 mice were splenectomized as described in Materials and Methods. Seven days later splenectomized mice and C57BL/6 control mice were either CD4+ T cell depleted or left untreated. Groups were then infected with 2 × 10^6 PFU of VSV (A1 and A2) or 2 × 10^6 PFU of VSV G (A3 and A4). Splenectomized and nonsplenectomized C57BL/6 mice were immunized with either 2 × 10^8 PFU of UV-inactivated VSV (B) or 500 μl of poliovirus i.v. (C). D, Splenectomized and nonsplenectomized C57BL/6 mice were CD4+ T cell depleted, and 5 × 10^6 PFU of VSV was injected in 20 μl directly into mesenteric lymph nodes. Ab titers were followed up to day 8 after infection. Neutralizing IgG on day 8 after immunization was at least two titer steps lower than total Ig, indicating that the Abs measured until day 8 largely represented neutralizing IgM. Experiments were repeated twice with comparable results. Results are given as the mean ± SD of three to five mice per group.

Splenectomy, therefore, did not block IgM production, but changed the mode of B cell activation from a TI Ab response to a TD Ab response (Fig. 3, A1–A4). The capacity to mount B cell responses independent of T help was not a unique feature of the spleen. Injection of 5 × 10^8 PFU of live VSV directly into the mesenteric lymph nodes of splenectomized and anti-CD4-treated animals primed B cells largely without T help (Fig. 3D) compared with Ab responses after i.v. infection of CD4+ T cell-depleted and splenectomized C57BL/6 mice (Fig. 3A2). In contrast to earlier observations with nonreplicating model Ags (37), our results indicate that all secondary lymphoid organs (lymph nodes or spleen) allow the induction of a TI Ab response if high amounts of Ag reach these tissues. However, under physiological conditions, only the spleen is able to efficiently filter Ag out of the circulation to trap sufficient amounts of Ag for a T-independent Ab response. In contrast, and importantly, comparable amounts of Ag injected s.c. apparently failed to reach peripheral lymph nodes, probably via dendritic cells (16), in a sufficient dose and adequate manner to activate B cells without T help.

Role of Ag structure

So far, our results indicate that TI Ab responses are determined by Ag dose and localization. Earlier studies have suggested a role of Ag structure (i.e., rigidly repetitive vs monomeric) on the induction of TI Ab responses (6, 12, 38). We therefore quantitatively compared the efficiency of VSV virions (exhibiting a highly organized, rigidly repetitive, neutralizing determinant on the intact virus) and VSV G protein preparation (where micelles show only a low degree of organization) in the induction of a neutralizing Ab response (Fig. 4A). The concentration of VSV particles in the used virus preparation was determined by electron microscopy. One infectious plaque-forming unit analyzed by a plaque forming assay on Vero cells corresponded to ~10 viral particles counted by electron microscopy (not shown). Since the number of glycoprotein per virion is about 1300 (1), the amount of VSV glycoprotein per plaque-forming unit of VSV could be calculated in micrograms of VSV glycoprotein. In CD4+ T cell-depleted mice VSV glycoprotein expressed on formalin-inactivated, and therefore nonreplicating, VSV particles induced Ab responses with 1000 times lower Ag doses than a recombinant VSV G protein preparation (Fig. 4A). For this comparison both Ag forms were injected directly into the spleen to overcome the possibility that VSV virus particles and VSV G protein might be filtered with different efficiencies to secondary lymphoid organs. These results confirmed that the structure of the Ag also plays an important role in the induction of TI Ab responses. However, in a viral infection, but also for active immunizations, as shown in Figs. 1 and 2, the infection route and kinetics seem to be the more critical parameters determining the early TI Ab responses.

Protective role of TI and TD Ab responses

In the infections and immunizations tested here, TI or TD Ab responses were induced with all infectious pathogens tested dependent upon immunization dose and route. What, then, is the biological relevance of TI Ab responses compared with TD Ab responses? VSV infection of mice causes paralysis and death if the virus reaches neuronal tissues (11, 39), and protection against a primary infection strictly depends on rapid generation of neutralizing Abs (9). We therefore analyzed the relevance of a TI Ab response in VSV infection. Splenectomized C57BL/6 mice, which cannot mount a TI Ab response (Fig. 3, A1 and A4), failed to control virus titer in the blood after an i.v. infection with 2 × 10^7 PFU VSV (Fig. 4B), had detectable infectious virus in the brain (Fig. 4B), and developed paralytic disease (Table I). In contrast, after a high dose infection (2 × 10^8 PFU) s.c., a route of infection that fails to systematically spread at high levels (Fig. 4B), but that also only induces a delayed TD Ab response (Fig. 1A), all mice survived (Table I). These results indicate that a TI Ab response is crucial for the organism to cope with rapidly replicating pathogens that spread via blood circulation at high titters.

Immunohistochemical analysis of TI and TD Ab responses

The implied importance of Ag trapping and accumulation in spleen after i.v. immunization was evaluated by immunohistochemistry.

The implied importance of Ag trapping and accumulation in spleen after i.v. immunization was evaluated by immunohistochemistry.
Infection with VSV offers the unique possibility that VSV Ag and VSV-specific B cells can be stained well in histological sections (25). Infection of mice with a high dose of VSV that elicits TI Ab responses (Fig. 1A) revealed the presence of Ag in the splenic marginal zone on day 2 after infection (after infection with 10⁸ PFU VSV; Fig. 5A). By 48 h after infection, B cell foci had formed around the splenic marginal zone (after infection with 10⁶ (not shown) and 10⁸ PFU of VSV; Fig. 5C); subsequently, specific plasma cells were observed in the red pulp (Fig. 5E). VSV-specific plasma cells are characterized by an intense intracytoplasmic staining, whereas VSV-specific B cells expressing the specific receptor mainly on their cell surface are only surface stained. After infection i.v. with 10⁴ or 10² PFU of VSV, doses that elicited TD Ab responses (Fig. 1A), VSV-specific B cells or plasma cells were observed only on day 6 and later (Fig. 5, D and F), and no Ag

![FIGURE 5. Immunohistochemistry of TI and TD Ab responses. C57BL/6 mice were infected with a high dose of VSV (10⁸ PFU (not shown) or 10⁶ PFU (A, C, and E) or with a low dose of VSV (10⁴ PFU (B, D, and F) or 10² PFU (not shown)). Spleen sections were stained for VSV Ag 2 days after infection with 10⁵ (A) or 10³ (B) PFU of VSV. Two days (C and D), 4 days (not shown), 6 days (E and F), or 8 days (not shown) after infection with 10⁴ (C and E) or 10² (D and F) PFU of VSV, spleen sections were stained for VSV-specific B cells and VSV-specific plasma cells. Two or three animals were analyzed per infectious dose and time point. One of two similar experiments is shown. Original magnification, ×100 (A and B) and ×400 (C–F).](http://www.jimmunol.org/)

### Table 1. Susceptibility of splenectomized and control mice to i.v. and s.c. infections with VSV

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<tr>
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a Splenectomized and nonsplenectomized C57BL/6 mice were infected with different doses of VSV i.v. or s.c., and survival was followed up to 30 days after infection. Results are shown as number of surviving mice vs total number of mice tested. Mice that developed hind leg paralysis were sacrificed according to the Swiss law for animal protection.

b Splenectomy was performed as described in Materials and Methods 1 wk before immunization.

![FIGURE 4. TD and TI Ab responses against VSV. Descriptive model for TI and TD Ab responses. C57BL/6 mice were either CD4 T cell depleted or left untreated. VSV particles in the virus preparation were counted by electron microscopy, and the amount of VSV glycoprotein per plaque-forming unit was calculated according to the fact that VSV particles possess 1300 glycoprotein molecules in their surface. Different amounts of pure VSV glycoprotein expressed either on formalin-inactivated VSV particles or as recombinant baculovirus-derived VSV G protein were injected intrasplenically into CD4⁺ T cell-depleted C57BL/6 mice (A). Ab titers were assessed on day 4 after infection of three mice per group. Results are given as the mean ± SD. The experiment was repeated twice with similar results. B. Splenectomized and nonsplenectomized control C57BL/6 mice were infected with 2 × 10⁹ PFU of VSV i.v. or s.c., and the virus in serum and brain was assessed at the time points indicated by a plaque forming assay. Results are given as the mean ± SD of four animals per group, and the experiment was repeated twice with comparable results. C. Model of the requirements (Ag dose vs amounts in the spleen, described as areas) to induce TI-1, TI-2, or TD Ab responses.](http://www.jimmunol.org/)
could be stained in the splenic marginal zone (Fig. 5B). The first appearance of VSV-specific B cells after a low dose infection occurred indeed largely only in T cell areas of the spleen where efficient T help can be provided (Fig. 5F). Note that by days 6–8 after infection comparable Ab titers were mounted independently of the infectious dose (Fig. 1), and comparable numbers of plasma cells were induced (Fig. 5, E and F) after a high or a low dose infection with VSV. Thus, B cell foci around the marginal zone seem to correlate with, and are probably a consequence of, early Ag trapping by splenic marginal zone macrophages and represent a histological correlate of an early TI Ab responses.

Discussion

An infectious pathogen that enters systemic circulation (viremia or bacteremia) would obviously be controlled most efficiently by preexisting, so-called natural, Abs (5). Since natural Abs have to deal with an enormous variety of possible Ag specificities (40), the serum titers are rather low. If this first line of defense is overrun by a high infectious dose or a too rapidly replicating pathogen reaching systemic circulation, then specific Abs must be produced rapidly to protect the host against cytopathic effects of infection. Since TD Ab production requires ~4–6 days to mount a specific Th cell response (21), the Ab kinetics may be too slow to protect the host from the consequences of a high-dose systemic infection. TI Abs can fulfill this early function efficiently.

Two interlinked parameters determine the induction of TI-1, TI-2, or TD Ab responses: 1) Ag dose and 2) efficient recruitment of Ag to secondary lymphoid organs (summarized as the model in Fig. 4C). Thus, the general rules for B cell induction are similar to those for T cells, where the amount of Ag present in secondary lymphoid organs over a given time period are the key to their induction (41, 42). The role of Ag structure in B cell induction is probably linked to both Ag amount and cross-linking of B cell receptors. The latter is only possible if a high Ag density is present on splenic marginal zone macrophages. Therefore, cross-linking of B cell receptors is achieved either by high amounts of monomeric Ag trapped on marginal zone macrophages leading to a high density of epitopes on their surface or by fewer multimeric repetitive particles leading to a focal, but also locally dense, concentration of Ag. Complement and natural Abs seem to be involved in Ag trapping and targeting to marginal zone macrophages (43). Marginal zone B cells have been shown previously to be involved in TI Ab responses (44, 45). Therefore, if the pathogen itself exhibits the neutralizing epitope in a repetitive form, B cell receptor cross-linking is mediated more easily, and the dose of Ag required in secondary lymphoid organs to induce TI Ab responses is lower. Nevertheless, after infections with highly repetitive Ags, an efficient Ag pooling to the splenic marginal zone is still the key to inducing a TI B cell response.

Most infectious pathogens enter the host via mucosal surfaces (46) and not at high doses directly via blood. Pathogens then spread to local lymph nodes and start a local immune response before systemic spread (2). The findings presented here offer an explanation for the increased susceptibility of splenectomized patients to selected systemic infections. In accordance with our viral models, patients after splenectomy, with functional asplenia (e.g., sickle cell anemia) or infants with an immature splenic marginal zone (47) are not able to mount TI Ab responses. As a consequence, they are susceptible predominantly to pathogens that are initially controlled on mucosal surfaces without inducing a potent systemic humoral immune response but that suddenly may lead to a massive hematogenic spread, i.e., *Haemophilus influenzae*, *Streptococcus pneumoniae*, and *Enteroviridae*. Additionally, these patients cannot be vaccinated with TI polysaccharide vaccines.

Of course, efficient B cell induction is also a goal of any improved design of vaccines. Virus-like particles have been shown to be one efficient means to elicit TI Ab responses (48, 49). Taken together, these and our results suggest that application of Ags at sufficiently high doses directly into secondary lymphoid organs or in formulations that will be efficiently trapped to and filtered out in secondary lymphoid organs should rapidly induce highly efficient protective B cell responses.

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References


