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Lymphocyte Polyclonal Activation: A Pitfall for Vaccine Design against Infectious Agents

B. Reina-San-Martín, A. Cosson and P. Minoprio

In this article, Bernardo Reina-San-Martín, Alain Cosson and Paola Minoprio summarize the marked alterations in the immune system functions after infection that might account for the poor success of effective parasite vaccine development. Many of the studies on oligoclonal B- and T-cell responses to parasite antigens aiming at vaccination strategies would seem to ignore more general, and perhaps fundamental, aspects of parasite-immune system interactions. In essence, because of its consequences on immunopathology and parasite escape, the authors ascribe a central importance in the pathogenesis of parasitic diseases to the 'nonspecific' polyclonal lymphocyte activation that occurs during infection. Hence, novel targets and strategies for immune intervention should be considered.

It is a common belief that parasites, viruses, fungi and bacteria often evade the immune system through adaptive mechanisms, that render the immune response powerless. Classic approaches to vaccine development have focused on the study of specific, parasite-directed mechanisms such as 'immunodominant', 'immunopathological' and 'protective' epitopes, in search of molecules able to trigger protective immunity while avoiding evasion. However, there is an important gap in this thinking: the immunologically relevant interactions between the infectious agents and the host are not limited to specific immune responses. Parasites, viruses, fungi and bacteria can actually obliterate specific immune responses by simply triggering the machinery of polyclonal lymphocyte responses, thus resulting in a general lack of specificity of antibodies or T-cell responses to the microbial antigens during infection and in the immunosuppressive state that follows. This apparently reduced availability of lymphocyte clones able to respond to the infectious agent (and to heterologous antigens as well) can actually be explained either by conventional immunosuppression or by the extensive engagement of most lymphocyte populations in effector functions that are not clonally specific (hyperstimulation). The onset of autoimmunity, another unwanted and frequent consequence of infectious processes, can

also be explained by the establishment of a long-lasting polyclonal activation, with the bulk of lymphocyte populations activated by the infection embodying host-directed cell clones involved in the evolution of self-aggressive mechanisms.

Vaccine strategies aimed at neutralization of mitogens/superantigens and the control of nonspecific responses are considered here; for additional references, see <http://www.pasteur.fr/recherche/unites/tcruzi/minoprio/PTrefs.html>.

Immune system-driven approach to infection

There are obvious difficulties faced by the immune system in order to eliminate a parasite. It is striking that a normal immune system is able promptly to reject tissues or organs that differ from the host by just a few amino acids in a single major histocompatibility complex (MHC) molecule. In contrast, the immune system is unable to eliminate parasites bearing a very complex and extremely different antigenic composition to that of the host. Nevertheless, the immune responses induced by parasite infections are sufficiently 'strong' to lead to progressive autoimmune pathologies that frequently can kill the host. Paradoxically, immune mechanisms that are inappropriate to eliminate the parasite are capable of destroying the host itself.

Infectious agents share the ability to activate a high fraction of total lymphoid cells, many of which differentiate to exhibit effector functions. The consequences of this quasi-panclonal activation of the immune system are: (1) the development of nonspecific B- and T-cell responses; (2) the immunosuppression of humoral and cellular responses to homologous and heterologous antigens; and (3) the onset of autoimmune processes that might arise from the expansion of self-reactive clones. The magnitude of these responses and the profound perturbation of immune homeostasis they bring about are major hindrances to the development of effective vaccine strategies. Thus, lymphocyte activation in infection is essentially the result of mitogenic and/or superantigenic microbial components that elicit relatively poor specific responses. However, classic vaccination approaches have attempted to 'neutralize' 'immunogenic molecules' that do not prevent panclonal activation and are, therefore, ineffective in controlling mitogen-dependent immune disorders and parasite evasion.

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In short, beyond the search for protection via the specific immune responses, the whole range of interactions between the infectious agent and its host immune system has generally been poorly explored. An additional difficulty of studies of infection and immunity comes from the conspicuous inability to correlate results *in vitro* with the complexity of the *in vivo* situation. In contrast to the 'parasite-driven' approaches described above, we have prioritized the consideration of the physiology of the immune system, with its autonomous activity¹ and characteristic dynamics, which are severely disturbed by the presence of an infectious agent, regardless of clonal specificity. This could be designated as an 'immune-system-driven approach'.

Polyclonal activation is characteristic of infections

Hypergammaglobulinemia, lymphoproliferation and induction of nonspecific and autoreactive antibodies are characteristic of polyclonal B-cell lymphocyte activation. These features have been described following various types of infection with viruses, bacteria and parasites²⁻⁴ (Table 1; D. Portnoi, PhD Thesis, Université Pierre et Marie Curie, Paris, 1984). In bacterial infections, this polyclonal activation was associated with the presence of mitogenic products: lipopolysaccharide (LPS) from Gram-negative bacteria, protein A from *Staphylococcus*, flagellin of *Salmonella*, polysaccharides from *Corynebacterium* and a variety of other mitogens and extracellular products^{5,6}. Thus, essentially all Gram-positive and Gram-negative bacteria possess mitogenic substances. Likewise, infection with Epstein-Barr, influenza, herpes and sendai viruses initiates non-specific B-cell stimulation resembling polyclonal stimuli^{7,8}; the same is described for non- or poorly pathogenic viruses such as lactic dehydrogenase and Theiler's. Furthermore, infection with murine leukaemia virus (MuLV-LP-BM5), which causes murine acquired immunodeficiency (MAIDS), is characterized by a severe immunodeficiency with abnormal lymphocyte proliferation, hypergammaglobulinemia and immunosuppression, resulting from nonspecific polyclonal activation of the immune system⁹.

Both T-cell-dependent and -independent polyclonal B-cell activation have also been described for a variety of protozoan parasite infections. In the case of *Plasmodium berghei*, *P. yoellii* and *P. chabaudi*, polyclonal B-cell activation does not seem to be solely the result of B-cell mitogenic stimulation, because lymphocytes from athymic mice not only significantly increase their ability to produce immunoglobulins, but both the magnitude and isotypic pattern of the response are different

Table 1. Immune dysfunctions observed after mitogen-induced polyclonal activation following infectious processes

Microorganism	Target lymphocytes	Acute or progressive dysfunctions ^a
<i>Actinomyces viscosus</i>	B	Immunosuppression
African swine fever virus	B	Immunosuppression
<i>Ascaris</i>	B	IgE secretion, allergy, cerebral granuloma
<i>Borrelia burgdorferi</i>	B	Autoimmune arthritis
<i>Candida albicans</i>	B	Granuloma formation, immunosuppression
<i>Chlamidia trachomatis</i>	B	Lymphocytosis, autoimmunity
<i>Entamoeba histolytica</i>	T	Immunosuppression, disabling colitis, liver abscess
<i>Escherichia coli</i>	B	Toxic shock syndrome, meningitis, neurological and systemic symptoms
<i>Leishmania donovani</i> , <i>L. major</i>	B	Immunosuppression, autoimmunity
<i>Listeria monocytogenes</i>	B and T	Meningitis, immune complex formation and immunosuppression
<i>Mycobacterium tuberculosis</i>	T	Immunosuppression and autoimmune arthritis
<i>Plasmodium chabaudi</i> , <i>P. yoellii</i>	T	Immunosuppression, autoimmunity
<i>P. falciparum</i>	B and T	Immunosuppression, autoimmunity
<i>Salmonella paratyphi</i> , <i>S. typhimurium</i>	B	Lethal septicemia, vascular myocardial injuries, immunosuppression, autoimmunity
<i>Schistosoma mansoni</i> , <i>S. hematobium</i>	B	Immunosuppression, megasyndromes, granuloma
<i>Staphylococcus aureus</i>	B	Toxic shock syndrome, mastitis, immunosuppression
<i>Streptococcus intermedius</i> , <i>S. mutans</i>	B	Toxic shock syndrome, immunosuppression
<i>S. pyogenes</i>	B and T	Immunosuppression, autoimmunity
<i>Toxocara canis</i>	B	Eosinophilia, lung damage, ocular granuloma, vasculitis
<i>Toxoplasma gondii</i>	B and T	Encephalitis, myocarditis, immunosuppression
<i>Trypanosoma brucei</i>	B	Immunosuppression, glomerulonephritis and brain lesions
<i>T. congolense</i>	B	Immunosuppression
<i>T. cruzi</i>	B and T	Hypergammaglobulinemia, immunosuppression, autoimmune myocarditis, megasyndromes

^aData from Ref. 4, and the additional references listed there at <http://www.pasteur.fr/recherche/unites/tcruzi/minoprio/PTrefs.html>

from that observed in euthymic mice¹⁰. Rather, it would seem possible that malaria parasites induce the (polyclonal) mitogenic activation and proliferation of T cells that, in turn, amplify and modulate B-cell responses¹¹. The polyclonal activation of $\gamma\delta$ T cells has been claimed to have a potential role in the maintenance of the B-cell hypergammaglobulinemia and complex cytokine network established after *P. falciparum* and *P. chabaudi* infections of human and rodents^{2,12}, as well as after human toxoplasmosis¹³. Similarly, cooperation was observed between T and B cells triggered by *Leishmania major* and *Trypanosoma cruzi*, where polyclonal lymphocyte proliferation and differentiation of effector B cells were determined in the absence of cognate recognition^{14,15}. Impaired cell-mediated immune responses in several host species were equally associated with B- and/or T-cell polyclonal activation induced by other parasites, namely *Toxocara canis*, *Ascaris* spp, *Entamoeba histolytica*, rodent and African trypanosomes, *T. cruzi* (reviewed in Ref. 2) as well as *Schistosoma mansoni* and *S. hematobium*^{4,16}.

Table 2. Immune dysfunctions associated with superantigen-induced polyclonal activation

Microorganism	Main common acute or progressive dysfunctions ^a
Bacteria and protozoa	
<i>Mycoplasma arthritidis</i>	Toxic shock syndrome, hypergammaglobulinemia, immunosuppression and autoimmunity (arthritis, nephritis, myocarditis)
<i>Staphylococcus aureus</i>	
<i>Streptococcus pyogenes</i>	
<i>Trypanosoma cruzi</i>	
<i>Toxoplasma gondii</i>	
<i>Yersinia pseudotuberculosis</i>	
Viruses	
<i>Cytomegalovirus</i>	T-cell induced B-cell polyclonal activation, reduction in diversity, anergy, megasyndromes, immunosuppression, neoplasia and autoimmunity
<i>Epstein-Barr virus</i>	
<i>Herpesvirus saimiri</i>	
<i>HIV</i>	
<i>Influenza virus</i>	
<i>MMTV</i>	
<i>Murine leukemia virus</i>	
<i>Polyoma virus</i>	
<i>Rabies virus</i>	
<i>Sendai virus</i>	

^aData from Refs 17, 18, and the additional references listed there at <http://www.pasteur.fr/recherche/unites/tcruzi/minoprio/PTrefs.html>

In addition to mitogens, other products have been proposed to explain the massive lymphocyte activation and the ability of the infectious agents to avoid the host-specific response and ensure survival: superantigens (Table 2). For instance, the superantigen staphylococcal enterotoxin B can interact with all T cells expressing a particular V β chain and directly stimulate a high number of T cells that, only by chance, would be 'specific' to the bacterial peptides. Superantigen-reactive T cells suffer phenotypic alterations that precede a state of hyperactivity, with high production of cytokines, followed by an 'anergic' state. These cells are then unable to proliferate upon stimulation, become tolerant and are programmed to die (via apoptosis). Microorganism-derived mitogens and superantigens can drive the immune response to the profit of the microorganism. Thus, a direct consequence of superantigenic stimulation is a reduction in diversity, immunodeficiency and immune evasion.

The complex antigenic composition of infectious agents might be taken as suggesting that polyclonal activation of B and T cells results from 'hyperstimulation' of lymphocyte clones specifically directed to the multitude of challenging antigens. However, this is unlikely because one would expect a better control of the initial phases of infection by these activated clones responding to each of the many infectious antigens. Molecular and cellular analyses of B- and T-cell repertoires responding to infections have indeed shown that the indiscriminate utilization of genes encoding B- and T-cell receptors is incompatible with oligoclonal responses. We have estimated that the proportion of specific clones in the immune response to parasite infection is relatively minor, varying from 2–5% of the global lymphocyte activity triggered by infection¹⁹. We have therefore, proposed that attention should be given instead to the other 95–98% of the immune response to infection. In essence, we consider that understanding

infection and polyclonal lymphocyte activation, through their consequences on immunopathology and parasite escape, is fundamental for a better understanding of the interaction between the microorganism and the host immune system².

Polyclonal activation, progressive diseases and microorganism persistence

The importance of polyclonal lymphocyte activation following infections can be evaluated by the broad range of disturbances presented by the infected host. Polyclonal hypergammaglobulinemia follows virtually all experimental and clinical infections and is intimately associated with increased numbers of cells in secondary lymphoid organs (splenomegaly and lymphadenopathy), reflecting the profound stimulation of the immunological apparatus. Although this puzzling amplification of the immune response could be viewed as a primitive defense mechanism²⁰, it is certainly a very ineffective process that, in addition, brings about the unwelcome production of autoreactive antibodies. In the case of autoimmune lymphoproliferative diseases, the failure to control B-cell reactivity, and the consequent production of autoantibodies of various specificities, stems from the breakdown of 'clonal contraction' as a result of intrinsic defects modifying lymphocyte sensitivity to activation-induced cell death²¹. Infections, even those caused by rodent trypanosomes, erroneously considered as 'nonpathogenic'¹⁶, invariably lead to the development of an intense lymphocyte cellularity that precedes hypertrophy of lymphoid tissues and marked immunosuppression. Thus, infectious agents could deliver anti-apoptotic signals to prevent clonal contraction, induce hypercellularity and ensure evasion and persistence in the host (ie. HIV-1, herpes, adenovirus, *Mycobacterium bovis*). Such mechanisms could also be invoked to explain the genesis of organ-specific or systemic autoimmunity²², in late stages of infection, as defects in apoptosis constitute an aggravation factor in the physiopathology of autoimmune diseases. Considerable evidence is available that correlates infectious processes with the initiation of autoimmunity, or else with the precipitation of host-tissue aggression. One classic example is the acceleration of the onset of systemic lupus erythematosus (SLE) by LPS from Gram-negative bacteria²³. The hypothesis of molecular mimicry between microbial and host antigens is often invoked to explain post-infectious autoimmunity. Although this has been proved in a few cases, and sequence homologies between bacteria, viruses and host molecules are frequent²⁴, it should be underlined that microorganism-driven polyclonal lymphocyte activation is just as appropriate an explanation^{2,25}. Thus, as normal individuals contain autoreactive B cells, polyclonal responses necessarily include the activation of such clones and the production of autoantibodies, as has been shown in a variety of infections, and in model systems involving injection of microbial mitogens. Autoimmune myocarditis after *T. cruzi* and coxsackie virus infections has been attributed to both the stimulation of heart-directed T- and B-cell clones expanded during acute polyclonal lymphocyte activation and to the induction of specific antibodies crossreacting with heart and parasite antigens^{4,19,24,25}. The induction of polyclonal antibodies

to HIV molecules can also be related to the breakdown of tolerance and the development of autoimmunity²⁶. Furthermore, polyclonal immunoglobulin responses and superantigenic reactive, MHC class II-dependent CD4⁺ specific T cells are also involved in the hypergammaglobulinemia, auto-antibody formation and immune complex-mediated chronic proliferative arthritis caused by *Mycoplasma arthritidis*²⁷.

This polyclonal, mitogen/superantigen-induced autoreactivity has also been proposed in other parasite models, namely the autoimmunity induced by human and rodent malaria¹⁰, as well as by mycobacterium infections⁴. The similarities between the B-cell repertoire found in uninfected individuals, and the one exhibited after infection-driven autoimmune processes in general, suggests that systemic autoimmunity is overwhelmingly a consequence of the (hyper)stimulation of B-cell clones, rather than that of 'specific', but simultaneously autoreactive and parasite-directed clones. However, both polyclonal activation and molecular mimicry mechanisms are not mutually exclusive and could be combined to explain the induction of pathological autoreactivity induced by infectious processes²⁸.

Paradoxes for vaccination approaches

We have investigated the mechanisms and consequences of polyclonal lymphocyte activation using the experimental model of Chagas disease, caused by the protozoan parasite *T. cruzi*. As in other infectious processes, this disease involves extensive B- and T-cell activation, hypergammaglobulinemia and the establishment of chronic autoimmunity affecting the heart and the digestive tract^{2,14}. *Trypanosoma cruzi* infection induces a lymphocyte blast transformation of a magnitude that is similar to or higher than that induced by the classic polyclonal activator LPS¹⁴.

The truly polyclonal nature of the response to *T. cruzi* parasites was demonstrated by the indiscriminate representation of V_H gene families expressed by responding B cells²⁹. Thus, *T. cruzi* infection leads to a broad utilization of all known V_H gene families, whose pattern resembles that observed in the compartment of B cells naturally activated in normal, uninfected animals. In addition, the parasite infection stimulates the production of antibodies presenting the same type of reactivities as that of natural (anti-self) antibodies¹⁹. Interestingly, most of these multireactive IgM and IgG antibodies do not bind to parasite molecules but recognize autologous proteins. Data derived from unselected hybridomas isolated from infected animals show that most B cells activated in the acute and chronic phases of infection fail to recognize parasite antigens and thus are 'parasite nonspecific'. *Per se*, these findings could be used to explain parasite evasion.

Immunosuppression to homologous and heterologous antigens has also been established in Chagas disease, and interpreted as a consequence of the polyclonal responses. We have assumed that the immune reaction occurring early after infection determines, at least in part, the outcome of the late phases of the disease. The fact that a large fraction of lymphocytes is activated at late phases of disease, when parasites are rare, suggests that alterations in immunoregulatory mechanisms take place in the

primary infection, allowing the autonomous maintenance of hyperactivity of the immune system to contribute to pathology. The expansion of self-reactive clones, abundantly represented after infection, might be related to the autoimmune pathology.

The correlation between nonspecific responses and the negative consequences of infection (immunosuppression, persistent infection and susceptibility to immunopathology and autoimmune phenomena) is supported by increasing evidence showing that resistance to infection and immunopathology are also correlated with a reduction of immune system activities. We have shown that X-linked immunodeficient (Xid) mice are highly resistant to *T. cruzi* infection and to the characteristic immune pathology of susceptible mouse strains³⁰. This defect, caused by a point mutation in the gene encoding Bruton's tyrosine kinase (B + K), results in a severe B-cell immunodeficiency. These mice display very limited polyclonal B- and T-cell activation after *T. cruzi* infection, and most enter the chronic phase of the disease without signs of severe cardiopathy. The general principles we have concluded from these original observations were later supported by the finding that Xid mutant mice were resistant to microorganism-induced pathologies in models such as *L. major*, *Staphylococcus aureus*, *Trypanosoma brucei*, *Schistosoma mansoni* and MuLV infections³¹. Furthermore, the Xid immune defect can inhibit the spontaneous development of autoimmunity – namely the SLE-like syndromes characterized by hypergammaglobulinemia and antibody-dependent pathology³².

Similarly, the genetic or somatic depletion of cell populations in susceptible strains can lead to a better prognosis in several infectious processes. Some examples include the relative resistance to *S. mansoni* and *L. major* after *in vivo* antibody treatment with anti-CD4, attenuated tissue pathologies in *T. cruzi* and *Listeria monocytogenes* infections in mice genetically deficient in $\gamma\delta$ T cells, and the prevention of cerebral complications of rodent *P. berghei* malaria after *in vivo* anti-leukocyte function antigen (LFA)-1 antibody treatment³³. Interestingly, low T- and B-cell reactivities are fundamental to the establishment of protective immunity and resistance to *Streptococcus mutans* infection and pathology³⁴. In short, these observations reveal a paradox for current vaccination strategies; namely that resistance seems to be associated with reduced, rather than enhanced, immune activities.

Reconsidering vaccination approaches

The correlation of polyclonal lymphocyte activation of the immune system after infections with poor specific responses and severe immunosuppression to autologous or unrelated challenges should be taken into account when considering vaccination strategies. As discussed above, in several, if not all, models of infection, persistence of the microorganism and the immunosuppressive state brought about by infection correlate with the intensity of nonspecific polyclonal activation. Although many mechanisms have been proposed to explain this state of 'refractoriness' or failure to respond to external challenges, we suggest that, this is essentially the same question as that of the 'class determination' of immune responses^{2,34}, commonly referred to as 'Th1 (T helper type 1)- or Th2-polarized

T-cell response'. Thus, immunosuppression is installed in the face of intense lymphocyte (effector) activity that must be of the 'inappropriate' class, at least for parasite elimination purposes. Furthermore, resistance to different infections requires different classes of lymphocyte responses, depending on the infectious agent. It is important to consider that if vaccinating products prime individuals for the unsuitable class of response, we could be faced with the problem, already claimed by other colleagues, of 'vaccinating for disease'³⁵, or at least, of inducing unwanted immunosuppression or even associated autoimmune pathology.

In general, two types of vaccine strategies have been used to approach immunity to infection: one utilizes attenuated, or inactivated, microorganisms. If, as recently suggested by work with bacteria³⁶ and previously noted by Arala-Chaves *et al.*^{3,34}, attenuation of infectivity or pathogenicity is related to the loss of mitogenic, polyclonal activities, the success of this type of vaccine gives strong support to our views and this observation raises interesting questions. An infection where nonspecific microbial mitogenicity, and therefore polyclonal response, is reduced will be dealt with successfully by the normal immune system, avoiding immunosuppression and all the deleterious effects of the immune reactivity discussed above. Thus, this type of vaccine would exclude neutralizing responses towards the mitogenic activities of the infectious agent, so leaving the individual unprepared to face the natural challenge. Success would then rely on a degree of attenuation that is compatible with specific, neutralizing responses to mitogenic moieties.

The second strategy for vaccination seeks to utilize microbial 'immunodominant', 'immunopathological' and 'protective' epitopes that would induce the most effective immune responses to reduce the microorganism load (clinical immunity, or prevention of symptoms in spite of infection). Epitope-based rationales for vaccination result, in principle, in an immune response directed to the relevant epitope on the pathogen and therefore do not prevent any other toxicity associated with other epitopes. In this case, however, given that natural infection systematically leads to immunosuppression, the approach necessarily requires that vaccination should result in a state of complete, 'sterile' immunity, ie. prevention of infection. Thus, if vaccinated individuals are nevertheless susceptible to the initial phases of a natural challenge, the effects of previous vaccination (even if it has resulted in increased abilities to produce 'neutralizing' responses) are jeopardized by the immunosuppression that rapidly follows natural infection. Successful subunit vaccines to date are based on the neutralization of, for instance, bacterial toxins by the induction of 'specific antibodies'. Tetanus and diphtheria are good examples. However, protective immunity is still a major goal for intracellular pathogens whose neutralization relies on T-cell-mediated mechanisms (eg. *Mycobacterium*, *Listeria*, *Salmonella*, *Toxoplasma*)³⁷. Subunit vaccines for these microorganisms would not necessarily be a good choice if they do not contain adequate T-cell epitopes to fulfill the criteria of protection and induction of memory cells³⁸. Again, if the 'sterility' is not achieved upon challenge, the beneficial effects of the vaccine are likely to be abrogated.

In the case of parasite infectious processes, 'sterile' vaccines have not been described so far and it would seem difficult to elaborate epitopic vaccines that would result in the long-term maintenance of effector-specific immunity (ongoing antibody production or continuous generation of effector T cells), necessary to ensure 'sterility' upon infectious challenges. In other words, although vaccines aim at inducing immune states of specific 'memory', the prevalence of polyclonal activation/immunosuppression in infections requires that conventional vaccines (epitope-specific) induce a permanent effector immune response. In general, current approaches to elicit protective immunity do not seem to accord the necessary attention to these crucial aspects of parasite-immune system interaction and to the general rules of the immune responses to infection.

We have previously proposed a shift of emphasis in vaccine strategies, advocating the 'neutralization' of mitogens/superantigens and control of nonspecific responses, thus allowing for the dominant operation of protective specific immune mechanisms. This program requires the characterization and isolation of mitogenic, or 'adjuvant-like' moieties, on which to base the development of vaccine preparations capable of inducing specific antibody responses to such determinants. Therefore, this alternative goal for vaccination would aim at inducing specific neutralizing antibodies directed to parasite molecules implicated in the initiation of nonspecific (polyclonal) immune responses. It is worth recalling that classic 'antigens' stimulate B-cell clones that are mostly 'specific' to the inducer molecule and possess little effect on other lymphocyte clones. In contrast, 'mitogens', like the prototype LPS, stimulate both the proliferation and the differentiation of several B-cell clones, regardless of specificity³⁹. Thus, mitogens are very 'immunogenic' without being 'antigenic'^{5,6,40}. Consequently, the antibodies induced after mitogenic stimulation recognize very few, or none, of the inducer molecules. Induction of specific responses by mitogens is indeed possible, but is inversely correlated to the injected dose: low doses of mitogens induce specific B-cell responses to epitopes on the mitogenic molecule, while at high doses, the response is polyclonal and accompanied by a selective paralysis of specific B cells⁴⁰. Vaccination protocols that aim at neutralizing mitogenic and/or superantigenic activities of the microbial agent seem possible and, to be successful, would re-establish immune system homeostasis, thus allowing the development of specific, 'conventional' clonal responses to microbial antigens and a reorientation of the immune system to specific mechanisms of parasite clearance. Our proposal is compatible with a 'two-stage' vaccination protocol, where the first would necessarily aim at neutralizing 'mitogens' and the second, other targets, would be used in the same line as done conventionally.

In conclusion, it seems that a systematic and global analysis of immune activities during infection – rather than of the specific responses – is necessary for a better understanding of all mechanisms involved in infection. Experimental models of resistance/susceptibility might be helpful in this respect, for they might unravel evolutionary strategies that can then be exploited to reorient the immune system and establish protective immunity, without unwittingly initiating progressive pathologies. We propose that the induction of

immune protection necessarily requires: (1) a better comprehension of common mechanisms used by parasites, bacteria, viruses and fungi to induce polyclonal lymphocyte activation, notably the isolation of the molecules that are responsible for these effects; and (2) vaccine strategies capable of neutralizing or inactivating such molecules and/or activities in incoming infectious microorganisms, such that specific responses are allowed to develop and persist in the host.

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Program for Monitoring Infectious Diseases (ProMED).

The global electronic reporting system for outbreaks of emerging infectious diseases, a program of the International Society for Infectious Diseases, has a website at <http://www.promedmail.org>, maintained as a public service, where you can find current alerts and summaries for the last 24 hrs and also details of how to subscribe to their excellent list server ProMED-mail.

Genamics Journalseek. This claims to be the largest journal information database on Earth. At <http://genamics.com/journals/> it provides information on abbreviated titles, links to online site, availability of abstracts/full-text online etc. – and even journal impact factors.