V-REGION-RELATED AND -UNRELATED IMMUNOSUPPRESSION ACCOMPANYING INFECTIONS

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This paper discusses current evidence for the relationship between polyclonal lymphocyte activation, specific immunosuppression with decreased resistance, and autoimmune pathology, that are all often found associated with infections by a variety of virus, bacteria and parasites. The central question of class determination of immune effector activities is considered in the context of the cellular targets for nonspecific mitogenic activities associated with infection. A model is presented to integrate these findings: mitogens produced by the microorganism or the infected cells are preferentially active on CD5 B cells; the resulting over-production of IL-10 will tend to bias all immune activities into a Th2-mode of effector functions, with high titers of polyclonal antibodies and little or no production of gamma IFN and other "inflammatory" lymphokines that often mediate resistance. In turn, these conditions allow for parasite persistence and the corresponding long-term disregulation of self-directed immune reactivities, resulting in autoimmunity in the chronic phase.

This model would predict that selective immunization with the mitogenic principles involved in deregulation, could stand better chances than strategies of vaccination based on immunopotentiation against other, functionally neutral antigenic epitopes. It is argued, however, that the complexity of immune responses and their regulation, together with our ignorance on the genetic controls of class-determination, offer poor prospects for a scientifically-based, rational development of vaccines in the near future. It is suggested that empirically-based and technologically developed vaccines might suceed, while basic scientific approaches are reinforced and given the time to provide a better understanding of those processes.

Key words: lymphocytes – polyclonal activation – class regulation – autoimmunity

It is an old observation that individuals infected by virus or unicellular parasites, mount diminished responses to unrelated antigens. Several decades ago, it was also demonstrated that endotoxin, while constituting a strong adjuvant of antibody responses when administered simultaneously with antigen, "suppressed" specific immune responses to subsequent antigenic challenges over the next few days. Many alternative interpretations have been put forward to explain such infection-associated immunosuppression. We shall here briefly review our own work in this area, and propose a general model that accounts for some of the available observations.

IMMUNOLOGICAL CONSEQUENCES OF INFECTION

Infectious processes in man and experimenal animals provoke various immunological effects, only one of which is usually considered – the *specific immune responses* to antigens of the microorganism. Invariably, however, infection is also accompanied by the so-called *nonspecific immune response,* and by *immunosuppression* as well. Finally, a very common condition observed in the chronic phase of various types of infections, is the establishment of aggressive *autoimmunity.* Much of our work has been concerned with the correlation between these other components of the immune reactions to infection and the respective mechanisms, attempting to determine the influence these variables might have on the resistance or susceptibility to infection.

It should be underlined that, while the specific immune reactions are the most extensively studied, particularly in what concerns vaccine development, nonspecific responses are, quantitatively at least, far superior. Furthermore, it would appear that such other consequences of infection are to be considered within the context of vaccination strategies, if the hypotheses are confirmed that they have a bearing on the final outcome of resistance or susceptibility. Finally, if the autoimmune processes are triggered, at least in part, by such nonspecific reactions, this may pose serious questions. Thus, depending upon the vaccinating material and the particular condition under study, it could be as dangerous to vaccinate as to infect, that is, the probability of precipitating an autoimmune disease could be significant.

THE COMPLEXITY OF THE DETERMINATION OF IMMUNE RESPONSES

Hen egg white lysozyme is perhaps the most well known of all model antigens. Thus, several lysozyme-antibody complexes have been crystallized and their fine structure analyzed, covering almost the entire surface of the antigenic molecule; nearly every lysozyme peptide has been cleaved or synthesized and studied in various mouse strains, for T cell recognition, priming or recall abilities; transgenic mice have been produced to study various mechanisms of B and T cell tolerance; yet, we ignore thus far the basic rules leading to the clonal dominance of T cell responses to lysozyme in inbred mouse strains. This sobering remark should be considered when discussing perspectives of protective vaccination, and that model system be compared to the extremely more complex antigenic mixtures of a parasite, that replicates and undergoes various types of molecular evolution in the human host. Moreover, parasites, and other microorganisms as well, replicate in the host, and produce all sorts of biologically active molecules that interfere with important functions in the host itself.

This leaves some of us with little hope for a rapid success of the scientific approaches to vaccine development, notably in what concerns anti-parasite vaccines that are clearly the most difficult to achieve. Unfortunately, the history of the last hundred years seems to confirm our pessimism. It turns out, indeed, that the rate of introduction of antimicrobial vaccines in clini-

cal practise has not changed at all from early in this century. Everyone knows that the most "important" vaccines were produced before we ever knew about T and B cells, let alone antigen processing or MHC presentation, and that the heavy investments in novel "biotechnology" approaches have had so far a very limited impact. It would seem, therefore, that the empirical, purely technological and "inventive" pathway of development may well be the fastest to reach our current goals.

THE POLYCLONAL LYMPHOCYTE RESPONSES TO INFECTION

Every antigenic challenge results in the production of antibodies that fail to bind to the corresponding antigen and are thus referred to as "nonspecific". Many alternative models have been proposed to explain this phenomenon, from isotype-specific helper T cells, to "mitogenic" properties of the antigens, and to "network" compensations. We shall not review them here, but only indicate that such processes show an unusually high magnitude in essentially all infections that we have studied. In murine *Trypanosoma cruzi* infections, for example, up to 50% of all T and B cells in the spleen are activated to blast transformation, and mice undergoing a primary *P. chabaudi* contain up to 200 millions splenic cells in mitotic cycle, as compared to some 5-10 millions in uninfected controls. These overwhelming levels of lymphocyte activation lead to effector functions of at least a fraction of the activated cells; thus, the numbers of high-rate immunoglobulin-secreting cells of both IgM and IgG classes increase up to 100 fold each, and various kinds of effector T cell activities have been scored (cytotoxic, helper and suppressor). Interestingly, at least in conditions where chronic tissue infection persists, relatively high levels of polyclonal lymphocyte activation are observed throughout such late stages, even in the absence of parasitemia. Similar observations have been recorded in viral, bacterial and fungal infections as well, such that we take the above conclusions as prototypic of all infections. It should perhaps be underlined that, in normal mice, the invariably dominant immunoglobulin isotype in such responses is IgG2a, followed by IgG2b.

THE NONSPECIFIC NATURE OF THE LYMPHOCYTE RESPONSES

The mechanisms bringing about polyclonal responses have been amply debated, many pro-

posing that they merely represent the sum of all specific responses to the multitude of parasite antigens. We have addressed this question in several ways. The fact that all VH-gene homology families participate in the murine B cell response to *T. cruzi* has first shown its truly polyclonal nature, while its nonspecificity was ascertained by the failure of more than 95% of unselected monoclonal antibodies, derived from acutely infected mice, to bind to parasite surfaces or intracellular antigens. A more quantitative approach was taken to analyse the specificity of the primary B cell responses to *P. chabaudi* infection: B cell blasts generated in response to infection were cloned by limiting dilution, and their reactivities tested against a panel of antigens, including whole parasite extracts; in parallel, the frequencies of the same set of reactivities was determined among immunoglobulin-secreting plasmacells induced by infection. The conclusions were quite clear: there is no preferential stimulation of B cell clones directed to parasite antigens, which are simply part of the polyclonal response, together with other self and nonself antigens.

We conclude that most (if not all) of the lymphocyte activities in the acute phase of experimental murine malaria infection are driven by mechanisms that are largely independent of the V-region specificity of the responding cells.

CLASS DETERMINATION OF THE IMMUNE RESPONSES

Some 20 years ago, Parish had established the inverse correlation between antibody titres and DTH reactions in response to a large range of priming or challenging antigen concentrations. Recently, this major problem of class determination in immune activities has been brought back to the limelight, through the description of two major functional types of effector CD4 T cells, namely inflammatory and helper. These two classes of CD4 T cells produce a distinct range of interleukins, and have been physically separated either by cloning, or by the differential expression of surface markers (in the rat, for example). The relative concentrations of interleukins produced by either type of effector CD4 cells, together with intrinsic characteristics of the antigenic ligands, also determine the classes of antibodies produced in antigen-specific responses in several model systems. It has been known for

many years indeed, that with very rare exceptions, the class of antibody responses is not epitope-dependent, but rather determined by the "carrier". Interestingly, however, the polyclonal antibody response to primary infection is always IgG2a and IgG2b in normal mice, although it might differ in secondary challenges or in immunodeficient animals (see below). Finally, when finding activated CD8 T cells devoid of cytolytic effector activity, which could very efficiently perform in the suppression of B cell responses and showed requirements of recognition and cell interaction that could not be distinguished from "conventional" CD8 effector cells, we have proposed that two functional classes of such cells also exist.

The problem has acquired a particular importance in the context of anti-infectious defence, because of the work in several laboratories, pioneered by J. Louis and colleagues, showing that specific CD4 "inflammatory" responses to *Leishmania* are protective, while the corresponding "helper" type responses actually facilitate the progression of infection. Moreover, the genetic control of resistance versus susceptibility in that case, seems to essentially operate through that type of class determination of immune responses. The differential abilities of "helper" or "inflammatory" CD4 cells to provoke the destruction and elimination of the parasites cannot be separated from corresponding differential participation of the two cell types in tissue destruction and autoimmune pathology. Thus, as shown by Powrie and Mason, the transfer of "inflammatory" CD4 T cells to syngeneic nude recipients induces multiple, organ-specific autoimmunity, while "helper" CD4 T cells from the same donors, not only reconstitute nude hosts without signs of autoimmune disease, but they actually "neutralize" the pathogenic activity of the "inflammatory" cells.

These questions on the class determination of immune responses are, therefore, doubly important for our concerns here, both in what respects elimination of the microorganism and the autoimmune pathology of the chronic phases of infection. To complicate the whole issue, it is very likely that resistance to infection requires the differential participation of different classes of response, depending on the particular microorganism in question. Unfortunately, our current knowledge on the rules of class determination is very limited indeed,

TABLE

Mitogenic proteins secreted by microorganisms

		Streplococus intermedius	Streplococus mulans	African Swine[a] Fever virus	Candida albicans
Molecular weight		90 KD	38 KD	36 KD	43 KD
Isoelectric point		4.25	4.75	3.85	4.25
	Intact mice	+++b	+++b	++++b	++++b
B cell	Tx mice[c]	+d	+d	+d	+d
Mitogenicity	CD4+ depleted mice	?	+	+	+
in vivo	CD8+ depleted mice	++	?	+++	?
Specific	Intact mice	+++	+++	++++	++++
immuno-	Tx mice	+	+	+	?
suppression	CD4+ depleted mice	?	+	+	?
	CD8+ depleted mice	++	?	++	++
B cell mitogenicity in vitro		++++	++++	-(TNF?)	-(TNF?)

a: in this case, it remains to be established whether this is a viral or a host protein produced upon viral infection.
b: IgG2a and IgG2b are the predominant Ig classes in every case.
c: mice thymectomized as adults.
d: IgM and IgG3 are the predominant Ig classes in Tx mice.

such that pertinent criticisms have been raised on attempts to vaccination before we can control this central aspect. Obviously, if vaccination "primes" for the inappropriate class of response, it will be worse than no intervention at all, and we will be confronted with the possibility of "vaccinating for disease", as recently epitomized by others.

IMMUNODEFICIENT MICE THAT DEAL BETTER WITH INFECTION THAN IMMUNOCOMPETENT ANIMALS

It seems rather straightforward that, if the "wrong" class of immune responses may facilitate infection and/or autoimmune pathology, treatments that immunocompromise the host may lead to better clinical outcomes. This notion is quite frontally contradictory to conventional wisdom that has always assumed (and attempted) to indiscriminately "stimulate" immune reactivities to combat infection. In our own experience in a variety of infectious models, adult thymectomy, treatments with depleting concentrations of anti-CD4 or anti-CD8 monoclonal antibodies, a "germ-free" status, even the congenital absence of thymus, have often shown prolonged survival of the hosts, and sometimes, markedly decreased levels of infection, as compared to control animals. Invariably, however, two parameters of the immune response to infection are reduced,

namely the polyclonal lymphocyte response and the immunosuppression (see Table). This has suggested to us that polyclonal responses and immunosuppression are intimately associated, and both are, either causally related to a reduced resistance, or else, are induced by the same mechanisms that contribute to reduce host's resistance. In this case, the polyclonal response could be used as an "indicator" of those mechanisms, whatever they might be.

A characteristic of the polyclonal B cell/ antibody responses to infection is their marked dependence on CD4 T cells, that we assume to be of the "helper" class; the differentiation to effector functions of CD8 T cells seems to follow the same general rule. From the very nature of the "inducer" activity of "helper type" CD4 T cells, it would be possible to assume, therefore, that the above correlations between the magnitude of the polyclonal response, the level of compromise of the immune system, and the degree of resistance to infection, were all related to the levels of helper CD4 cell activity. Some observations in B cell-deficient mice came to support this interpretation and to lead to a general hypothesis. Having noticed the preferential activation of CD5 B cells in T. cruzi infected mice, we have studied the significance of this finding, challenging mice that

had been depleted of that cell type by adult irradiation followed by syngeneic bone marrow transplantation. We observed in these animals a near complete abrogation of the polyclonal response, that could be surprisingly reconstituted by the transfer of T cells from normal syngeneic donors. These observations have first called our attention for the fact that depletion of CD5 B cells seems to have effects on the functional behaviour of T cells, decreasing total levels of "helper" activity in the animals. Infection of *Xid* immunodeficient mutants, however, gave even more clear-cut results. These animals lack CD5 B cells, contain only about half of the normal numbers of B cells, and low circulating immunoglobulin concentrations. Yet, upon infection by *T. cruzi*, they develop low parasitemias under conditions that kill about 50% of the control, immunocompetent mice, the other 50% developing 10-100 fold higher parasitemias that are cleared only after 4-5 weeks. Moreover, *Xid* mutant mice, show essentially no signs of tissue pathology in the chronic phases of infection, notably in skeletal muscle, heart, and peripheral nerves, that are all markedly altered in control mice. As expected, *Xid* mice mount very reduced polyclonal responses associated with infection, but we do not yet know their degree of immunosuppression.

The resistance of these mutants is intriguing, particularly in the light of our assumptions that CD4 helper T cells are the pivotal lymphocyte class regulating the various responses associated with infection. Thus, *Xid* mutant mice are described as essentially normal in the T cell compartment. Recently, however, O'Garra and colleagues have reported that, in normal mice, the production of IL-10 is issentially restricted to CD5 B cells. Since IL-10 seems to be a major determinant in imposing a "helper type" pattern of CD4 T cell responses (by inhibiting "inflammatory" T cell proliferation, IL-2 and γIFN production), and since *Xid* mice lack precisely, CD5 B cells, it could be envisaged that the response of mutant mice was simply geared into the inflammatory type by deficient IL-10 production. The deficit of IL-10 responses upon infection of these mice has been directly shown, and on-going experiments provide further direct evidence for this assumption, by manipulating the mutant and normal "phenotypes" with appropriate treatments with IL-10 or the respective specific antibodies.

A GENERAL MODEL TO EXPLAIN POLYCLONAL RESPONSES, IMMUNOSUPPRESSION, AND DECREASED RESISTANCE TO INFECTION

The molecular and cellular mechanisms underlying the polyclonal responses accompanying various types of infections are not established. Considering that immunosuppression and autoimmunity, let alone susceptibility versus resistance, might be correlated with the magnitude of those responses, this question is obviously relevant. This is all the more so, if the mechanisms stimulating the polyclonal response are causally related to the control of the class of immune activities in the host. In what concerns bacterial infections, most would say that B cell mitogens are likely to participate in the process; thus, besides endotoxin, a number of other B cell mitogenic substances — lipoproteins, peptidoglycans, etc. — have been isolated from both gram-negative and gram-positive bacteria. Other possibilities exist, however, such as the mitogenicity of bacterial products to T cells, as is the case for exotoxins with "superantigen" activities, which stimulate large subsets of CD4 T cells, at least. The problem is, however, the heterogeneity of the situations where such responses are recorded. However, the generation of mitogenic substances for lymphocytes in infected individuals, by the microorganism or by the host cells themselves, might not be exceptional. We have undertaken to analyse a few cases, where B cell polyclonal responses had been demonstrated, by the isolation of substances with a mitogenic effect for B lymphocytes (Table). As can be seen, in this heterogeneous group of infections studied thus far, mitogenic substances could be isolated. Interestingly, both mitogenicity and immunosuppressive activities co-purified in every case. Moreover, an intriguing similarity in apparent molecular weight and isoelectric point were noticed. Other microorganisms are now under investigation, and the already isolated substances are being further analysed biochemically. Finally, the same and other microbial products are being tested for T cell mitogenicity of "superantigenicity".

We take, therefore, as a starting assumption that all infections result in the production (by the microbe, or by the infected host cell) of lymphocyte mitogens. If we now postulate that the preferential targets for those B cell mitogens are CD5 B cells, it follows that IL-10 production will necessarily be abundant in those infections. In turn, excess IL-10 will

impose a "helper type" effector function of the concomitantly activated T cells. which will lead to further recruitment and activation of other B lymphocytes and other classes of T cells as well. These effects are certainly facilitated and/ or amplified by the participation of putative T cell mitogens or "superantigens", the toxicity of which is essentially due to T cell activation, as recently much discussed by Kappler, Marrack and colleagues. The mitogenic activities, together with a predominant IL-10 component, will result in the massive polyclonal responses observed, with little effect on the microorganism itself. If the clearance of the infectious agent is not antibody dependent, resistance to infection will be further compromised, for the inflammatory reaction will be markedly suppressed. The persistence of the microorganism in the tissues, perhaps together with autoreactivities included in the polyclonal response, will thereafter "break tolerance" to self, and result in autoimmune pathology. Finally, under these conditions, it is not surprising that the immune responses of the infected animal to third-party antigenic challenges are severely compromised, and the old notion of "suppression by too much help" finds here a most appropriate example. All lymphocyte classes are probably responsible for the establishment of such immunosuppressive state, and have actually been described to mediate suppression in one experimental system or another (see also Table), notably, B cells, CD4 and CD8 αβTCR T cells, and γδTCR T cells as well.

This general model would suggest that current strategies of vaccine development are not necessarily correct. Thus, whole inactivated microorganisms, which, depending on the class of response necessary for protection in that particular case, may well induce immunity in some cases, are perhaps more likely to amplify the negative sides of the immune reactions discussed here. On the other hand, vaccination with a particular epitope from the microorganism, is not necessarily a good alternative either. Thus, if the immunity achieved is not "sterile", the whole cascade of events described above will be initiated upon reinfection, and are likely to abrogate the beneficial effects of previous priming. The success of "epitope" vaccines, therefore, requires one of two things, that might be difficult to achieve: sterile immunity, or mechanisms of "vaccinated" resistance that are refractory to the immune perturbations introduced by the

challenge, notably those which impose a given class of response and immunosuppression. An alternative possibility, directed suggested by this model, would be to "vaccinate" for antibody production, exclusively against the molecules that play a key role in initiating all those perturbations, namely the (CD5)B cell mitogenic substances. It is reasonable to expected that neutralization of the biological activity of such molecules will abolish or greatly diminish the particularities of infection-associated immune responses, and thus, will turn the problem into a "conventional" type of immune responses. In what concerns secondary immunosuppression and autoimmunity, at least, that type of "vaccine" seems likely to bring some new possibilities. Direct experimentation has been initiated along these lines, and very encouraging results are already available in what concerns resistance to *C. albicans* in mice.

OTHER TYPES OF MECHANISMS ESTABLISHING INFECTION-ASSOCIATED IMMUNOSUPPRESSION

A last comment on V-region-associated immunosuppression induced by infection, allows us to touch upon its most dramatic example, namely that of AIDS. Some years ago, we have proposed that the immunodeficiency associated with infection by HIV-1 is the result of the immune response to viral infection, rather than the consequence of the direct cytopathic effects of the virus. Since then, a number of similar proposals have emerged, all submitting that there is today enough information on the virology, immunology, epidemiology and clinics of AIDS, to conclude that the disease (the CD4 T cell deficiency) is essentially autoimmune. The theme is of great importance to discuss, when so many groups in the world are ready to initiate vaccination trials with normal volunteers. Thus, if there is some truth in those hypotheses, some forms of vaccination may actually induce the disease (CD4 lymphocytopenia) in uninfected individuals. We shall not discuss in any detail the arguments leading to such conclusions, but we will summarize observations obtained in the study of mice infected with Lactic Dehydrogenase Virus (LDV), which may be revealing of similar mechanisms.

LDV is a murine RNA virus with a very benign clinical course in acute infection, but a persistent, life-long viremia. The cellular receptors of this virus have been identified as

Class II molecules, precisely the complementary structures to CD4 molecules, that are the cellular receptors of HIV-1 in man. Furthermore, Coutelier, Van Snick and colleagues have previously characterised a very marked polyclonal antibody response to LDV infection, essentially in the IgG2a class, as in all other cases of infection. Finally, a considerable number of reports had established in the past that chronically infected mice are markedly immunodeppressed some months after infection. Given this starting information, and the availability of recombinant, soluble CD4 molecules, that facilitate assays of antibody specificity, we have investigated the basis of immunodeficiency in the chronic phases of infection. One thing more is interesting with the model, namely that T lymphocytes are never infected by LDV, for they do not express Class II MHC molecules in the mouse. The experiments consisted in measuring the recruitment of B cells secreting anti-CD4 autoantibodies to the polyclonal response, by either single-cell secretion assays or serum levels. The results show that a large fraction of all B cells activated in primary infection secretes relatively low affinity anti-CD4 autoantibodies which reach very high circulating titers along the first 3 weeks of infection. Up to 10% of all IgG2a-secreting splenic B cells display a detectable reactivity with CD4 molecules.

Although cause-effect relationships with circulating anti-CD4 antibodies are yet to be established, 2-3 months later, the CD4 T cell counts start decreasing in peripheral blood and, by 6 months of infection CD4 T cell numbers are only 25-30% of controls in lymph nodes and spleen as well. Moreover, the IgG2a fraction from the serum of infected mice show marked inhibitory effects for CD4 T cell responses in vitro, suggesting that indeed the anti-CD4 autoantibody production, stimulated together with the polyclonal response, does have marked suppressive effects. As LDV and HIV-1 use homologous, complementary molecules as receptors, if in both cases autoantibodies included in the polyclonal response are the mediators of immunosuppression, it could be anticipated by comparison that, in the case of HIV-1, anti-Class II antibodies should be the major cause of disease. This of course suggests therapeutic interventions for correcting immunodeficiency, which are now being tested in the model system.

REFERENCES

As this paper is a quasi-direct transcription of a talk at the CEC-FIOCRUZ meeting, it contains in the text the references to other's work, as they were taken then. In any case, all these can be found in full-length in any of our previous publications.