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Antigenic Structure and the Induction of Different Immune Responses to Parasites

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Though a long-standing activity in immunology, the search for molecular correlates of variable immunogenicity has yet to uncover many ground rules that, because they have general validity, can be inserted with confidence into the text books. Not that the search hasn't been rewarding: recent insights on aspects such as antigen processing, antigen presentation and T and B epitopes, have made the topic particularly interesting. The contribution of antigen structure to other traditional determinants of immunogenicity such as route of injection, size, depot formation, phylogenetic distance ("non-selfness"), persistence, "intrinsic adjuvanticity", type of added adjuvant, etc. will hopefully also be clearer soon. One problem can be brought into sharp focus by the simple question - "what is a suppressor epitope?" Clearly, the answer will depend on one's views on suppressor T cells. Moreover, many well-worn questions remain unanswered: what antigenic features, if any, lead preferentially to induction of CMI versus antibody,

to IgA antibodies rather than IgG or IgE, and what impact do natural or pre-elicited antibodies, immune complex formation, or complement activation (1), have on the immunogenicity of antigens of different structure. One dilemma for the cellular immunologist looking for general principles is that which will always accompany the reductionist approach - deciding on the relevance of many in vitro phenomena involving single cell suspensions to events in highly organized lymphoid organs.

Recent progress in the analysis of immunogenicity has resulted from, (a) the availability of synthetic peptides (2) and portions of antigens from recombinant DNA expression vectors, with or without attached components such as β-galactosidase, (b) monoclonal antibodies for more precise definition of B cell-stimulating epitopes including the mimotope (and mirror image anti-idiotype antibody) approach to conformational epitopes (3), (c) cloned T cell lines for identification of T cell-stimulating epitopes, (d) the move away from hydrophobic chemical haptens such as DNP/TNP to peptides and other molecules more relevant to pathogens (e.g. 4) and (e) the capacity to engineer hybrid molecules through recombinant DNA and other techniques in a manner relevant to antigen presentation - e.g. provision of an anchor sequence to soluble proteins (5) and the synthesis of composite antigens such as conjugation of bacterial proteins to bacterial (meningococcal, pneumococcal and haemophilus) polysaccharides (6), lipids to proteins (7,8) and peptides to proteins. The latter is exemplified by the conjugation of a N-terminal 19 amino acid hydrophilic peptide of the pre-S2 region of HBV to the S gene product (i.e. hepatitis B particles from plasma) (9), as well as a C-terminal peptide of β-HCG (10) and a 3x4 mer of the **P.falciparum** CS protein (11) conjugated to bacterial toxoids for immunological disruption of fertility or malaria infection, respectively.

Additionally, the clear demonstration that specifically-reactive B cells are efficient antigen-concentrating devices and are centrally involved in

antigen presentation for elicitation of antibody production, has enabled several puzzling observations to take on new meaning. [The B cell that binds native molecules (conformational epitopes), and presents processed forms to T_H cells, comes under the influence of T_H mediators with subsequent production of antibody of the specificity involved in antigen binding. In this discussion T_H refers to $CD4^+$ cells involved in help for antibody production, macrophage activation and delayed type hypersensitivity as distinct from $CD8^+$ cytotoxic or suppressor T cells]. These puzzles include "original antigenic sin" and the failure of carrier preimmunization to enhance anti-peptide responses in a one host system. Much can now be explained by severe competition for antigen amongst B cells with different antigen-binding capacities (antigen receptor affinity), other antigen-presenting cells (APC), and pre-existing antibodies (12).

As judged by the list of invited speakers, there is no point here going into the nature of restricted recognition of protein antigens by T_H cells. This, together with the question of affinity of foreign peptides for class I and class II MHC molecules, and the structural aspects of T_H epitopes, will no doubt be addressed in some detail at this meeting. What is unlikely to receive as much attention is the recognition of non-protein antigens by T_H cells. This of course is a contentious issue that is highly relevant to leishmaniasis, at least.

There is good evidence that T_H cells see denatured proteins and oligo-peptides particularly well. However, there is also some evidence that they are fully capable of recognizing conformational determinants (13-16) and non-proteins such as carbohydrates (17,18). The notion that T_H cells have difficulty recognizing pneumococcal polysaccharide and other poorly digested carbohydrates is well founded (19) and is reinforced by the known effects of adding protein carriers to bacterial polysaccharide antigens to increase antibody levels, particularly T_H cell-dependent IgG production (6). Per-

sistent, non T_H cell-stimulating polysaccharide antigens with a high density of B epitopes are usually good inhibitors of antibody production (20,21). However, it is my impression that this topic took a dramatic turn when Robertson et al (17) demonstrated classical DTH reactions to carbohydrate antigens in calves recovered from salmonellosis provided a lipid anchor was added to the eliciting antigen. This strongly suggests that if the carbohydrate antigen is anchored into the membrane of antigen-presenting cells then MHC-restricted recognition by sensitized T_H cells can occur.

Our own interest in this question of presentation of carbohydrate antigens to T_H cells derives from the demonstration that a glycolipid antigen purified from **Leishmania major** by an anti-carbohydrate monoclonal antibody was able to protect mice against cutaneous leishmaniasis whereas the aqueously soluble carbohydrate derivative of this amphipathic glycolipid was not (22,23). There are several peculiarities about this system, e.g. total failure of subcutaneous immunizations, the potency of the adjuvant **C.parvum**, and great difficulties in demonstrating any net clonal expansion of protective T cells in protected compared with naive mice (24). The efficacy of the glycolipid is presumably related to its capacity to orient in the membrane of APC for induction and to be available on the surface of the infected macrophage as a target of T_H cell-mediated protection. Not only is the carbohydrate derivative(s) of the glycolipid nonprotective, there are indications that responses induced to this form of the antigen facilitate persistence of disease (23). No T cell lines are yet available to substantiate claims that T_H cells see carbohydrate epitopes in this parasitic disease although it is of some interest that periodate treatment of crude antigen is reported to ablate in vitro responsiveness of lymphocytes from patients exposed to **L.major** (25).

Aspects of antigenic structure and behaviour that are likely to be key ingredients in differential immunogenicity include: (a) the number (valency)

and density (20) of B epitopes, (b) the number and perhaps location relative to B epitopes, of T_H epitopes including the number of T_H epitopes (or T_C epitopes) per APC. On this point, B cells with high affinity receptors can compete well for soluble antigen and concentrate it for presentation to T_H cells whereas in the case of whole organisms (but see 26) uptake by macrophages may be the only way of ensuring many epitopes per APC and thus efficient presentation to T_H cells (12). Liposomes and ISCOMS may function in a similar way. (c) persistence/indigestibility/aggregation of antigen, and (d) tissue and membrane affinity.

Aspect (d) needs elaboration. Membrane affinity could be of several types - e.g. affinity for class II MHC molecules or receptors at the cell surface or partitioning into the lipid bilayer of the cell (see below). It is known that negatively-charged polysaccharides localize specifically to marginal zones and sinuses of spleen and lymph node, respectively (27). Differential effects of macrophages and dendritic cells have been demonstrated in DTH for example (28). We have previously emphasized the property of persistence of antigen in non lymphoid tissues in regard to allergenicity (29,30). Moreover, molecules with affinity for membranes (and in high doses) may be inhibitory (31), the phosphorylcholine epitope of some helminths perhaps being suppressogenic because of inhibitory effects on (B) cells that bind it in large amounts (32). I have discussed above the likely influence of lipids in glycolipids, and anchor sequences in proteins, on membrane anchorage for class II MHC restricted recognition by T_H cells. An affinity of peptides for class I and class II MHC molecules (33-36) will no doubt be covered elsewhere in this conference. Affinity for cell surface receptors, if the latter are laterally mobile in the membrane, is also likely to increase the probability of enforced association with MHC molecules for immune induction and expression.

A general high affinity of antigens for membranes (i.e. high lipophilicity) may reduce access of antigen to specifically-reactive B cells in particular and therefore be weakly immunogenic as isolated molecules. It may be worthwhile studying this aspect of "passive avoidance" of B cells and other APC in regard to the marked differences between BCG and PPD in induction of PPD sensitivity and between **L.major** organisms and released carbohydrates (EF) in anti-carbohydrate antibody production (discussed in 12). If antigens with high membrane affinity are dispersed widely over many cell types then lymphokine (e.g. γ-IFN)-mediated enhancement of class II MHC expression may further divert T_H cells away from B cells such that antibody production is further decreased. Such speculative events may explain some of the variable effects of mediators such as γ-IFN on antibody production. Affinity of molecules for intestinal epithelial, in particular the cells overlying Peyer's patches, should be examined for effects on induction of IgA responses. Of the large number of unknowns in mucosal immunology, one is the mechanism(s) underlying the very great differences in vaccination efficacy of living (that do not have to be invasive, e.g. 37) versus dead organisms administered by the oral route. Details of antigen presentation by living virus-infected cells, and differences from "killed antigen" preparations in induction of T cell responses, is likely to come under closer scrutiny with the use of recombinant viruses such as vaccinia virus (38,39) to deliver antigens to the immune system (± co-expressed products from inserted immunoregulatory lymphokine genes in the vaccinia-virus infected cell). The undeniable potency of irradiated helminth larvae as protective vaccines relative to killed antigen (or unattenuated organisms for that matter) in some systems remains largely unexplained and is a topic ripe for detailed immunoparasitological analysis in filariasis, schistosomiasis, lung worm infections, etc.

What then of suppressor epitopes? The notion came into being when suppressor T cells were all the rage with the evidence that T_S and proliferating T cells see non-overlapping epitopes on a protein antigen (40). It gained ground with demonstrations that "adding T_S epitopes" to other immunogens lead to suppression (literature cited in 16,41). Of course, discussion on the topic quickly becomes bogged down in debate on what is (or whither) suppression and until the whole question of specific T_S and active $CD8^+$ T cell-mediated suppression in vivo is resolved, there seems little point in pursuing suppressor epitopes further in this discussion.

Adding or including T_H epitopes to poorly immunogenic parasite oligopeptides and proteins has been moderately successful in reducing the number of low or nonresponder mouse strains - e.g. falciparum malaria CS peptides (42,43) and the T_H cell stimulating hapten azobenzenearsonate (44) to a schistosome glutathione S-transferase antigen (45). Many falciparum malaria proteins have an extraordinary structure consisting of tandemly repeated amino acid sequences (46), that, together with extensive cross-reactivities between repeats in different molecules and within molecules (47), seem tailor made for immune diversion - i.e. deviation of antibody responses along low affinity pathways rather than allowing an orderly maturation of high affinity responses (12,47). If this is one of the means of immune evasion utilized by **Plasmodium falciparum** then vaccination to promote high affinity antibody production on first contact with the parasite may have a very different effect on parasitaemia than might be predicted from the rate of development of protective immunity in an endemic area.

A discussion on variable immunogenicity of molecules is usually not far removed from one on the mode of action of adjuvants and antigen-delivery vehicles used to promote desirable immune responses following vaccination. There is little doubt that one of the recurring challenges for the immunoparasitologist will be to promote T_H cell responses to antigens of parasites

(48) particularly if a T_H cell-derived or dependent mediator is required for anti-parasite effector function (as distinct from the effects of T_H cell-dependent antibodies). Low antibody production to a subset of host-protective epitopes can generally be increased without too much difficulty by conjugation to undefined T_H epitopes (e.g. through complexing to acceptable T_H cell-stimulating carriers such as bacterial toxoids). Such antigen engineering may or may not provide significant B cell sensitization for triggering of memory responses on later challenge with the parasite (depending on the baseline frequency of T_H cells). However, if a sensitized population of T_H cells to a subset of host-protective epitopes is required (e.g. to antigen on the surface of an infected macrophage with subsequent activation of that parasitized cell) it is difficult to imagine an antigen-engineering strategy ($\pm$ adjuvants/vehicles, etc.) that will overcome responses that are defective for genetic reasons (e.g. self-tolerance, failure of interaction between T_H cell receptors, antigen and class II MHC molecules). Responses that are low for immunoregulatory reasons could be a different matter and may be more prone to intervention.

How to overcome genetically-based low or non-responsiveness in T_H cells? In particular, how to deal with the problem at the effector or therapeutic level - e.g. focussing T_H cells at the surface of infected cells for the three diseases under discussion at this meeting. It has been known for some time that mycobacterial products and synthetic derivatives are particularly effective in stimulating T_H cell responses in general (48-51). This approach will no doubt continue to be exploited, particularly if BCG can be developed as an expression system and vehicle to deliver parasite antigens (52,53). However, I would like to end this discussion by highlighting another approach that should be worth pursuing - namely the use of hetero-bifunctional activating anti-T cell antibodies (54,55) (and eventually, simple chemicals or drugs designed to simulate the activity of anti-T anti-

bodies). Antibodies directed to any T cell surface molecule that can activate the T cell are conjugated to a second antibody with specificity for any parasite-encoded antigen at the infected cell surface. The hybrid hybridoma technique is a variation of this and the first report using anti-parasite antibodies has appeared recently (56). This means of focussing T_H cells to an infected macrophage in an individual where specific recruitment is not occurring is only likely to work if the avidity of binding for infected macrophages is greater than the avidity of binding for T_H cells. Choosing antibodies with differing combining site affinity may ensure that the injected composite antibody is not lost through binding to a large number of T_H cells (or even a subset depending on the choice of activating anti-T antibody) prior to ever seeing the infected macrophage. If the anti-T antibody has low avidity binding as a monomer but the avidity is increased by concentration through the anti-parasite antibody at the infected macrophage surface then efficient focussing may be achievable. Time will tell whether this immunotherapeutic approach will be of value in overcoming deficiencies in effector T_H cell functions because of an inability of T_H cells to perceive T_H epitopes on antigens at the surface of infected cells.

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