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THE IMMUNE RESPONSE TO POLYSACCHARIDES: A VIEW FROM THE BRIDGE

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SUMMARY

A view on immunity and tolerance to polysaccharides as thymus independent antigens within the general framework of cell-cell interactions in the immune system is given.

A body of experimental data is given in support of the contentions and to provide a basis for potential use of this knowledge for new vaccine and diagnostic developments.

INTRODUCTION

The antigen-specific T and B cell receptors probe the world around. Each one of them very specific, but together covering a wide, vast region of antigenic specificities, like an eye exploring the environment. Many immunologists would say that in the process they distinguish self from non-self; some others would prefer to see the immunological world as one looking into itself. I regard this particular divergency as something like a non-issue: the polemic that need-not-be, and taking a stance at the very beginning I would say that the history of the immune system, both phylogenetically and ontogenetically defines not only how the system

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TABLE 1

DIFFERENTIAL PROPERTIES OF ANTIGENS AS SEEN BY T AND B CELLS

	<i>Recognition</i> in conjunction with MHC on cellular membranes	<i>Is recognised by</i> cell associated T-cell receptors	<i>Chemical nature</i> peptides or derivatised peptides	<i>Epitope</i> Mostly non- conformational
T cells	cell surface associated and free Not in conjunction with MHC	cell-associated receptor and free antibody	Proteins Nucleic acids carbohydrates etc.	Conformational and non- conformational
B cells				

alone or in association with MHC (Major Histocompatibility Complex) molecules, is that no T cell help, suppression or cytotoxicity can be generated specific for carbohydrates and there are few believable reports that state the opposite (2,13). Still the controversy has not been resolved and the question whether T cells, whatever their function, can recognise carbohydrate epitopes remains un-answered. They could exist, however, since there is nothing indicating that *a priori* carbohydrate epitopes cannot interact with class I or class II MHC proteins in a way recognisable by T cell receptors.

How then, can these antigens act as B cell immunogens in the absence of T cell help?

In Table 2 a summary is given of the most important characteristics of the TI antigens. It contains a definition (a necessary element) and describes some aspects of their behaviour. A more detailed account of these properties can be found elsewhere (27, 31, 40). the definition of TI antigen is a functional one: the properties of these antigens are such that they stimulate antibody production without requiring *antigen-specific* T cell help. This characteristic is probably important, since multiple repetitions of the same epitope seem indispensable for B cell stimulation (17,

TABLE 2

PROPERTIES GENERALLY ATTRIBUTED TO THYMUS INDEPENDENT (TI)
ANTIGENS, SUCH AS PURIFIED POLYSACCHARIDES

1. Immunoresponse characterised by lack of T-cell requirement (by definition).
2. Epitopes repeated many times along the polymer.
3. Relatively low rate of degradation in vivo.
4. Immune response to TI antigens appears late in ontogeny compared with thymus dependent (TD) response of the same specificity.
5. In the mouse the response is usually IgM, but many exceptions are known.
6. B-cell memory is poor, or absent.
7. T-cell regulation, when demonstrable, appears to be either non-specific or apparently involving idiootype determinants.
8. A group of TI antigens, classified as TI-1, by virtue of the response in CBA/N mice seem to bear the potential to stimulate a different sub-set of B cells Most purified polysaccharides are not immunogenic for these mice, hence they are classified as TI-2 antigens. (32).

TABLE 3
ANTI- α (1-6) GLUCOSYL SPECIFIC RESPONSE OF CBA MICE
IMMUNIZED WITH DIFFERENT DEXTRANS*

Dextran	M.W.	% anti(1-6) linkages	Antibody forming cells/spleen**			
			I	10	100	1000
B512	>10 ⁷	95	2300 (1.54)	61800 (1.28)	26700 (1.59)	603 (1.85)
T-2000	2 × 10 ⁶	"	24200 (1.57)	32900 (1.32)	18600 (1.12)	260 (1.12)
T-150	1.5 × 10 ⁵	"	1620 (1.44)	2590 (1.76)	7020 (1.40)	840 (1.39)
T-70	7 × 10 ⁴	"	1040 (1.61)	830(2.21)	2680 (1.06)	150 (1.50)
T-20	2 × 10 ⁴	"	>100	> 100	130 (1.26)	200 (1.48)
B1355	>10 ⁷	69	7500 (1.49)	9400 (1.52)	29900 (1.30)	9360 (1.27)
B1299	>10 ⁷	53	330 (1.89)	22((1.47)	310 (1.94)	
—	—	—	160 (1.22)			

*Direct PFC assayed on day 5 after i.v. injection of antigen.

**Geometric mean of five animals are recorded. Figures in parentheses indicate the standard error.

These results have been verified by T and B cell transfer into X-irradiated animals. Therefore, TI is not only a property of the antigen but also has to be defined in relation to the state of the immune system at the time of immunisation.

The potential of these observations for applied use could be considerable as the immune response to purified polysaccharides in humans has several features that resemble the response in rodent and it appears reasonably safe at the moment to call it thymus independent. TI responses mature relatively late in the ontogenetic development of the immune system with the unpleasant consequence that most purified polysaccharide vaccines are not particularly successful immunogens in infants. Meningococcal polysaccharides can be mentioned as examples (10).

I see adaptative value in this behaviour, since polymeric self TI antigens (autoantigens) might stimulate maturing B cell clones before the system completes its self-regulating immunological network; meanwhile,

TABLE 4
ISOTOPE DISTRIBUTION (%) ANTI DEXTRAN B1355 ANTIBODIES IN FIVE
BALB/C MICE (AVERAGE)

Isotype	%
IgM	91
IgG3	6
IgA	2
All others	1

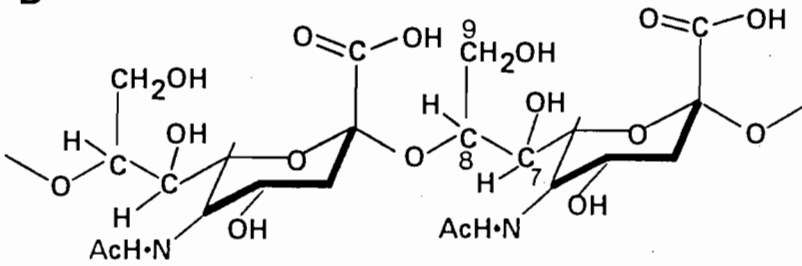
TABLE 5
ISOTOPE DISTRIBUTION (%) OF ANTI-MENINGOCOCCAL GROUP C
ANTIBODIES IN CBA MICE (5 INDIVIDUAL SERA)

	Serum				
	1	2	3	4	5
IgG1	23	47	90	25	13
IgG2a	22	8	7	25	23
IgG2b	9	23	0	10	21
IgG3	0	0	0	0	0
IgM	46	22	3	40	43

Repeating unit of meningococcal

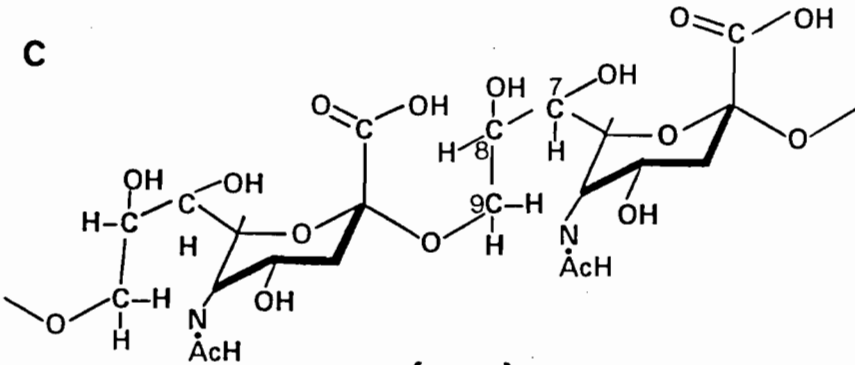
group B and group C polysaccharides

B



$\alpha(2-8)$ NANA

C



$\alpha(2-9)$ NANA

Fig. 3. The linkage (repeating unit) of N. Acetyl neuraminic acid in B and C capsular polysaccharides of *Neisseria meningitidis*.

TABLE 7
DETECTION OF SIALIC ACID POLYSACCHARIDES AND FOETAL RAT BRAIN
SIALOPEPTIDE WITH ANTI-B MONOCLONAL - BASED ANTIBODY COUPLED
TO LATEX PARTICLES

	Average Chain Length (NANA residues)	Minimum Concentration Detected ($\mu\text{g/ml}$)
<i>N. meningitidis</i> group B polysaccharide	200	0.015
Colominic acid	33	15
Colominic acid	18	20
Colominic acid	14	120
Foetal rat brain sialopeptide	15	60

de, it can be detected with the same monoclonal antibodies. Differentiation between K1 antigen and polysialidated brain proteins, or its products, only takes place under conditions in which the short chain of the latter warrants only a very weak, or negative reaction with the antibodies (see Table 7). Also in practical terms, and sighting vaccination, it means that purified capsular polysaccharides of *N. meningitidis* belonging to any group other than B can be used as vaccines in humans, whereas B polysaccharide is completely ineffective. The conjugation of short, $\alpha(2\rightarrow8)$ linked NANA residues to a protein carrier does not render them immunogenic either, indicating that it is not only a problem of T-cell helper activity. However, complexation of large molecular weight B polysaccharide with proteins does result in large amounts of IgG antibody since this polysaccharide is a short lived immunogen, the final stages of antigen-dependent B cell proliferation and differentiation are missing, and as a result the anti-B response is usually IgM and short lived (29).

Immune responses to polysaccharides, albeit not influenced in a specific way by T cell help can be boosted in a non specific fashion, as it is the case for the primary response to meningococcal polysaccharide group B. Fig. 4 illustrates the results obtained when mice were pretreated with killed *Corynebacterium parvum* followed at different intervals by a single, poorly immunogenic dose of meningococci group B. As seen in the figure a considerable increase in the anti-B response was observed even when *C. parvum* was given 20 days earlier.

The picture emerging from these, as well as from other studies, is that

via macrophage, non-specific T cell products are present the TI response switches to IgA possibly IgE production. Hence the description by some authors of IgA production to dextrans as TD (4). See Fig. 1. It also raises the questions: which interleukins, where are they produced and how do they regulate the immune response to TI antigens? So far, although there are several well characterised interleukins and a whole family of interferons that one way or another are capable of regulating the immune response (37), a review of the subject is not possible within the scope of this article. It is only pertinent to comment that continuous progress is being made in this area of research.

Lastly, the capacity of TI polysaccharides to induce antibody formation would depend on the localisation of them in the lymphatic organs and in different histological areas of the immune system. As it has been pointed out already (16, 17) this can be different according to the structure of the polysaccharide, the presence of circulating antibodies and the capacity of the polymer to stimulate the alternative pathway of complement. These factors play a role in the generation of memory cells (18, 23) and possibly in the induction and maintenance of tolerance.

TOLERANCE TO POLYSACCHARIDE ANTIGENS

As stated before (27) the effective interaction of antigen with the immune system could have two possible consequences: immunity or tolerance. This means that both immunity and tolerance (i.e. immune paralysis) require that the antigen engages the immune system and initiates changes that can be both positive and negative. Even when seen in the context of antibody responses, tolerance needs not be a B cell phenomenon since TD antibody responses have been shown to be affected by T cell suppression (8, 38). Moreover, direct induction of tolerance to helper T cells (7) will

TABLE 8
DEXTRAN-SPECIFIC TOLERANCE REVERSED WITH DEXTRANASE IN VIVO

Tolerogen (Day 0)	Dextranase Days 7 & 8)	Immunogen (Day 28)	Anti-dextran cells/spleen (standard error) (Day 33)
Img Dextran	+	1 μ g Dextran	3730 (2.16)
"	-	"	280 (1.35)
-	+	"	19480 (1.75)
-	-	"	15700 (1.75)

Spontaneous or induced break of B cell tolerance to a polysaccharide antigen (without clonal deletion)

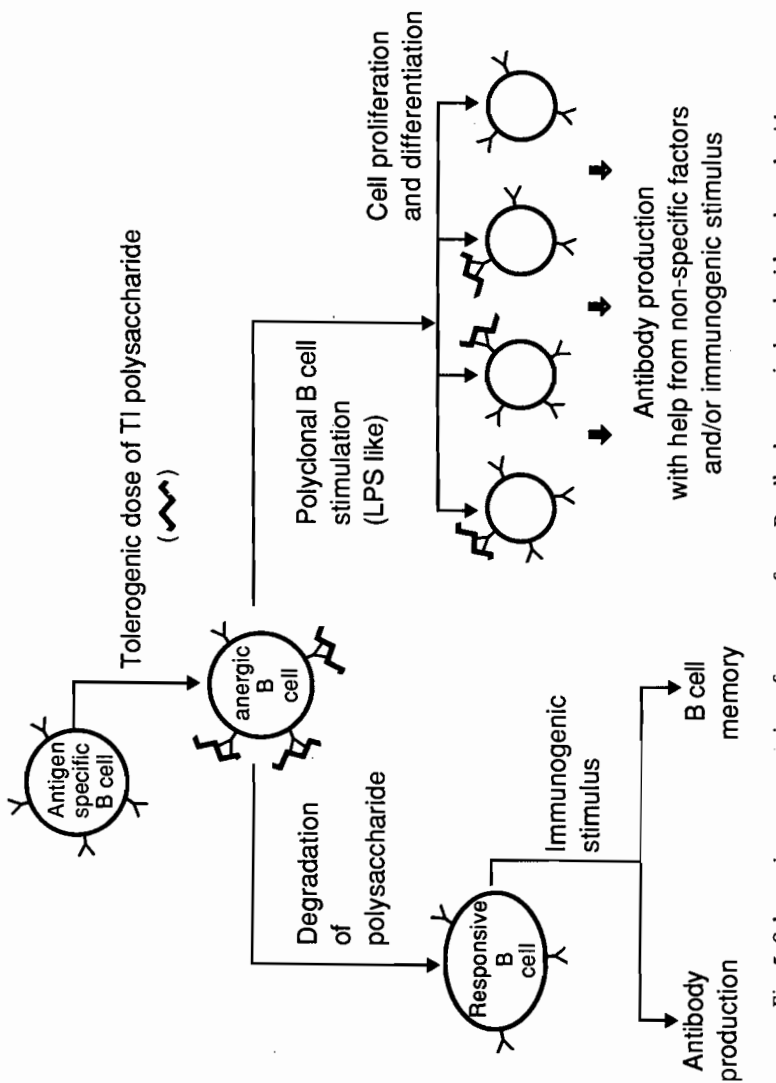


Fig. 5. Schematic representation of recovery from B cell tolerance induced with polysaccharides.

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