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## **Vaccination against encapsulated bacteria in humans: Paradoxes**

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## **Abstract**

Infection with encapsulated bacteria can be prevented by vaccination with capsular polysaccharides, either plain or conjugated to a protein carrier. But results concerning these vaccinations raise several paradoxes. Polysaccharides from encapsulated bacteria are generally considered to be T-independent antigens unable to trigger a T-dependent germinal center reaction, but strikingly, anti-polysaccharide antibodies are often mutated in humans. Polysaccharide-protein conjugate vaccines are able to induce a true T-dependent memory response with a rise in antibody titers and a switch to high affinity-IgG antibodies in children below 2 years of age, but neither the plain nor the conjugate vaccine can induce memory in older infants and adults. We propose some explanations to these paradoxes based on our recent observation that humans display a circulating splenic marginal zone B cell population with a pre-diversified immunoglobulin repertoire in charge of the immune response to T-independent vaccines.

The impact of diseases generated by encapsulated bacteria has been underlined in many contexts and it is estimated that they are responsible for millions of children's deaths each year [1]. Nevertheless, when looking at the abundant literature on immune responses to encapsulated bacteria after vaccination by either plain or conjugated capsular polysaccharide vaccines, some paradoxes emerge which we would like to discuss.

### **Paradox 1**

It is generally accepted that bacterial capsular polysaccharides (CPS) are TI antigens that trigger specific B cells residing in the splenic marginal zone to secrete antibodies with opsonophagocytic properties against these bacteria [2]. In rodents in which these responses have been mostly studied, immunization do not induce an extensive proliferation within B cell follicles, neither the formation of germinal centers, and are for most of them taken care by unmutated germline antibodies [3-5]. In

humans on the contrary, the splenic marginal zone contains B cells with mutated Ig receptors [6-8] and mutated antibodies are raised in responses to encapsulated bacteria, these mutations being responsible for the antibodies avidity for the immunizing antigen [9, 10]. In all species, these TI responses do not trigger any memory, the B cells engaged being able very rapidly to switch isotype and to secrete large amount of antibodies. These plasma cells can remain in the organism for various lengths of time, after which the response wanes out [11, 12]. To account for the presence in humans of mutated antibodies during a T-independent immune response that cannot normally trigger a cognate T-B dependent germinal center reaction, the classical explanation put forward by authors is that such responses are in fact taken care by *bona fide* memory B cells that have been primed by a previous encounter with the pathogen, either during an infection or by silent carriage [13-15]. These memory B cells would then eventually reside in the splenic marginal zone where they could acquire marginal zone B cell surface markers [16-18]. This explanation requires that during this natural priming which must have occurred in each case analyzed so far, the polysaccharidic capsules from the pathogen are somehow linked to a protein moiety in order to drive the response into the classical germinal center-dependent memory B cell pathway.

## **Paradox 2**

Children below 2 years do not respond to plain CPS vaccines but they do respond when the CPS are conjugated to a protein carrier (Table 1 and Box 1). Moreover these conjugate vaccines will induce a memory response with a rise of antibody titer after several rounds of immunization and a booster response several months later [21-24]. Surprisingly, in older infants and in adults, the same conjugate vaccine does not induce a memory response but a response similar to the one obtained with the plain CPS preparation [25-28], even though the response to the conjugate appears in some cases more robust [29-30].

## **A proposed explanation**

We have shown that humans display a circulating marginal zone B cell population which is involved in TI response and which diversifies its Ig receptor in the absence of cognate T-B interactions. These conclusions are based on the following experimental facts:

- 1) Hyper-IgM patients with either CD40L or CD40 deficiency do not have germinal centers and possess a population of circulating IgM<sup>+</sup>IgD<sup>+</sup>CD27<sup>+</sup> B cells with a mutated Ig receptor, in the complete absence of switched memory B cells. In both types of patients the frequency of Ig mutations observed in these cells is similar to the one observed in controls, although the number of IgM<sup>+</sup>IgD<sup>+</sup>CD27<sup>+</sup> blood cells is in general lower in the patients as compared to normal children. The absence of CD40 or CD40L may alter B cell development by hampering the network of cytokines interactions involving dendritic cells, activated T cells and natural killer cells. Nevertheless, the fact that these patients never display any CD27<sup>+</sup> switched B cell indicate that the IgM<sup>+</sup>IgD<sup>+</sup>CD27<sup>+</sup> B cells present are not produced in some cryptic germinal center structures, but rather belong to a separate population.
- 2) Phenotypic analysis and gene expression profiling in normal individuals indicate that human splenic marginal zone B cells are, as opposed to rodents [31], recirculating in the blood.
- 3) After vaccination with a non-conjugated pneumococcal vaccine, blood and splenic IgM<sup>+</sup>IgD<sup>+</sup>CD27<sup>+</sup> B cells are specifically mobilized in this T-independent response. This response is taken care of by B cells carrying mutated antibodies prior to immunization. Upon expansion of these cells in the splenic marginal zone during the week following vaccination, the hypermutation process is reactivated.
- 4) The blood IgM<sup>+</sup>IgD<sup>+</sup>CD27<sup>+</sup> B cell population starts to expand after birth. It is well expanded and mutated in children around one year of age, an age at which toddlers do not respond to T-independent antigens, thus strongly suggesting that diversification results at this stage from a developmental program and not from an immune response.

Our conclusion from these preliminary investigations is, as already proposed in our first report [32], that this so-called memory IgM<sup>+</sup>IgD<sup>+</sup>CD27<sup>+</sup> B cell population, which represents 10-30% of B cells in blood and spleen, belonged to a different pathway of differentiation and was linked to the response against TI-antigens [33]. A similar conclusion was reached by R. Carsetti and her colleagues in a recent report, based on their study on the responding capacity asplenic or splenectomized patients to T-independent pneumococcal antigens [34].

The model we propose offers obviously another solution to paradox 1 without having to involve an obligatory primary infection or carriage. The B cells involved in the response are already pre-diversified and can undergo another round of hypermutation after stimulation by the immunizing antigen. These B cells, which are already in a pre-activated state, do not possess any properties attributed to memory B cells. However, being constantly triggered by TI antigens, they could account for most of the so-called « natural » IgM antibodies present in the circulation [35].

In order to explain paradox 2 we would like to propose that before two years of age, in the absence of a functional splenic marginal zone, CPS vaccines conjugated to a protein carrier will trigger naive follicular B cells and drive them into a germinal center-dependent reaction including affinity maturation and memory B cell generation. After two years, the now functional splenic marginal zone will sequester polysaccharidic preparations from encapsulated bacteria, whether they are conjugated to a protein carrier or not, thus explaining why a similar marginal zone type of response without any memory characteristics is obtained with both the plain and the conjugated CPS vaccines. Strikingly, bone marrow transplanted patients, which cannot respond to pneumococcal CPS until approximately 2 years after the transplant, respond to the conjugate vaccine like normal toddlers below 2 years by generating a clear memory response [36, 37]. Thus in both situations in which the splenic marginal zone is not functional, do we see an uncoupling between the response to the plain and the conjugated CPS preparation.

There may be direct clinical consequences to our propositions that can be tested. Effectively, patients undergoing a splenectomy are usually vaccinated by the plain or the conjugated vaccines

prior to the operation. According to our scheme these patients should mount, like toddlers, a memory response if vaccinated by the conjugated vaccine after splenectomy. The same result should be obtained with asplenic patients.

Moreover, having observed that the B cells engaged in these responses are already well developed and mutated in blood at an age at which children cannot respond to the plain CPS vaccine, and taking into account that it will be challenging to conjugate the serotypes of all encapsulated bacteria (e.g. there are 90 serotypes for *Streptococcus pneumoniae* and only 11 have been conjugated at this moment), it is tempting to speculate that plain vaccines in conjunction with the appropriate adjuvant specific for these cells may be able to trigger a protective response in toddlers below 2 years. It is clear that such a result if possible would be a life-saving progress in underdeveloped countries.

There remain many unsolved questions concerning these cells and the response against encapsulated bacteria. The splenic marginal zone being obviously a complex lymphoid niche, is there a contribution in humans, even minor, of *bona fide* memory B cells to TI responses? What are the cells that sequester CPS antigens after 2 years, whether there are in their natural or vaccine form? Does this occur in the splenic marginal zone or also in blood through specific DC which can bring them to the spleen, and what are the specific receptors involved? [38, 39]

How and where do marginal zone B cells diversify their Ig receptor throughout life? During the first two years of life in infants one can study blood  $IgM^+IgD^+CD27^+$  B cells as they develop and diversify their Ig receptors, prior to any immune response. Thereafter encounter with external TI antigens in the mature environment of the splenic marginal zone will trigger their effector function, i.e. the production of antibodies. What pre-activates these cells and drive their final differentiation remains an open question. This pre-diversification step could take place in the splenic marginal zone and in marginal zone-like regions in lymph nodes and Peyer's patches [40]. It could alternatively occur in isolated B lymphoid follicles along the gut, as has been described for other species [41] (see below). A similar pre-activated stage has been described for NK T cells [42], and,

similarly to them, pre-activation of marginal zone B cells could be driven by self or bacterial commensal antigens. For both these cellular populations, NKT and marginal zone B cells which are intermediate between the innate and adaptive immune system, this pre-activated stage may explain why they react very rapidly upon challenge by external bacterial antigens [12].

### **Looking at other B cell immune systems**

GALT (gut-associated lymphoid tissues) species, as opposed to bone marrow species, do not use ongoing rearrangement in the bone marrow to generate B cell diversity. They include a large number of mammalian species studied today (sheep, rabbits, cattle, pigs, etc...) and have been named so because they generate their B cell pre-immune repertoire by post-rearrangement diversification processes such as hypermutation and/or gene conversion in gut-associated lymphoid tissues [43, 44]. This pre-diversification step is antigen-independent although gut bacterial antigens provide the mitotic stimuli permitting the intense proliferation of these cells in GALT follicles [45, 46]. Moreover, in all these species there is only one window of B cell production and diversification that occurs during several months after birth, the peripheral B cell compartment being then installed for the whole life of the animal. The obvious advantage of this mode of generation of the B cell repertoire by gene conversion and hypermutation, which target all three CDRs, is the variety of specificities and affinities produced, allowing these species to also respond to T-independent antigens with a large range of binding capacities.

Despite obvious differences between GALT B cells and human marginal zone B cells, our data imply that humans have most probably conserved for one arm of their B cell system the strategies used by GALT species to diversify their pre-immune repertoire. This strategy which differs from the one used by follicular B cells, allows the generation of a robust immune response against life threatening infections by encapsulated bacteria.



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Figure legend.

Figure 1. A proposed scheme for the response to PS vaccines, plain or coupled to a protein carrier. Round shapes represent B cells and irregular ones, cells involved in antigen capture (macrophages, dendritic cells,...).

### **Box 1: History**

“It occurred to us that if the pneumococcus polysaccharide could be combined with a foreign protein it should be possible to produce a conjoined carbohydrate-protein antigen capable of stimulating the formation of type-specific pneumococcus antibodies in the animal body. Provided the specificity of the original carbohydrate has not been too greatly altered either through chemical manipulation, or through the introduction of new molecular groupings, one should obtain on immunization with such a “synthetic antigen”, antibodies which would be identical in specific action with those produced by immunization with the intact bacterial cells.”

Walter F. Goebel and Oswald T. Avery *J. Exp. Med.* **54**, 431-436 (1931)

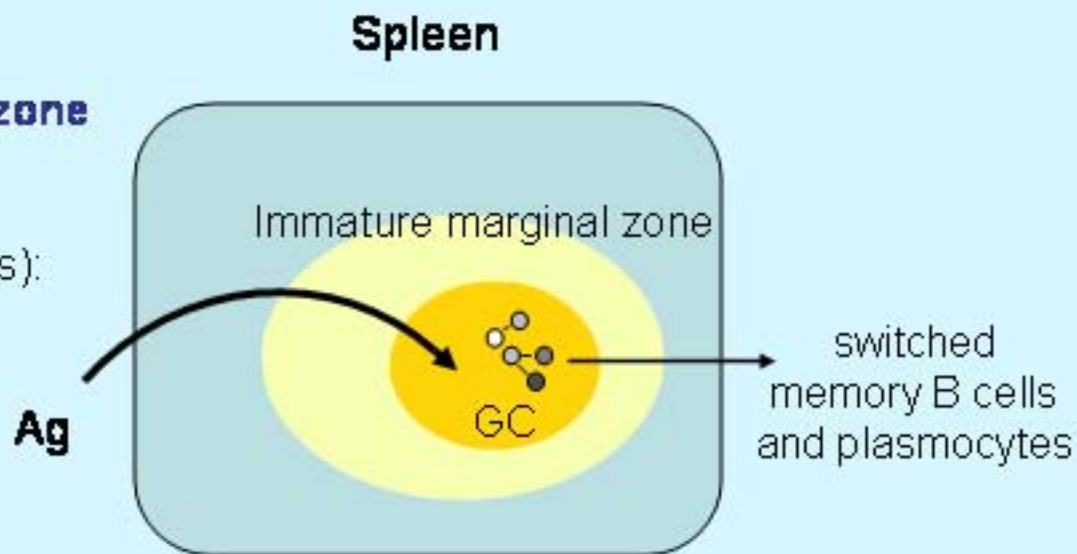
The first conjugate vaccine was developed by Goebel and Avery [19]. In trying to understand how the polysaccharides of the pneumococcal capsule were involved in the virulence of the organism, Avery a few years later identified DNA as the support of genetic information, in the famous bacterial transformation experiment with Colin M. McLeod and Maclyn McCarty [20].



Figure 1

**Immature / absent splenic marginal zone**

(children below 2 years,  
bone marrow transplanted patients/  
splenectomized and asplenic individuals):  
antigen response to protein-coupled  
PS-antigens in germinal centers:  
memory response



**Functional splenic marginal zone**

PS-antigen (plain or coupled) trapping  
in marginal zone:  
immediate T-independent response,  
no memory response

