Lifelong protection against hepatitis B: the role of vaccine immunogenicity in immune memory

Jangu Banatvala a,*, Pierre Van Damme b, Stephan Oehen c

a Department of Microbiology, John Radcliffe Hospital, Oxford, UK
b Centre for the Evaluation of Vaccination, Department of Epidemiology, University of Antwerp, Antwerp, Belgium
c University Hospital Zurich, Institute for Experimental Immunology, Zurich, Switzerland

Received 22 March 2000; received in revised form 5 June 2000; accepted 27 June 2000

Abstract

Long-term protection against hepatitis B (HB) disease is dependent on persistence of a strong immune memory. This paper presents and discusses new knowledge that allows a better understanding of the mechanisms of long-term protection following hepatitis B vaccination. Studies have revealed links between specific lymphoproliferation, the in vivo humoral response and immune memory. The strength of immune memory and of a subsequent secondary immune response can therefore be predicted by the antibody response following primary vaccination. Vaccine antigen dose and structure have been identified as important influences in the primary antibody response and development of immune memory. The data and considerations presented support the use of highly immunogenic HB vaccines in order to provide long-lasting protection against HB disease. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Hepatitis B; Vaccine immunogenicity; Immune memory

1. Introduction

More than 2 billion individuals worldwide have evidence of past or current infection with the hepatitis B virus (HBV), and there are over 350 million carriers of the virus, many of whom will develop chronic liver disease, including cirrhosis and hepatocellular cancer [1]. Chronic HBV infection is responsible for the death of around 1 million people each year.

Prevention at an early age is crucial in controlling the chronic carriage of HBV disease [2], hence the World Health Organization issued recommendations in 1991 that hepatitis B (HB) vaccine should be integrated into national infant and adolescent immunization programmes in all countries by 1997 [3,4]. Currently, over 110 countries have adopted national or regional policies of universal infant or adolescent immunization.

Achieving long-lasting protection to HB is an important goal for the individual, the physicians and nurses who administer vaccines, and for the public health and vaccine policy-makers. Long-lasting protection will ensure that high levels of immunity are maintained within the population, as well as eliminating any immediate or future need to introduce routine booster vaccinations. The resultant cost-savings associated with long-lasting protection arise from reduced morbidity and mortality in individuals who would otherwise be susceptible to disease, reduced costs of treating disease and, of course, reduced costs of booster vaccinations.

As clinical data on the longer term efficacy of HB vaccine is becoming available and data on the relationship between vaccine immunogenicity, cellular immunity and immune memory is accumulating, there is an opportunity to identify the factors that influence immune memory in HB disease. In this review, we discuss:

- studies on the relationship between immune response to HB vaccine and HBV infection and lymphoproliferation;
- evidence for immune memory to HB disease following vaccination and how this relates to the longevity of protection against this disease;
general studies on the role of antigens, in B and T cell immune memory, providing amore detailed understanding of the HB immunological study findings and demonstrating the interplay between vaccine immunogenicity, cellular immunity and immune memory;

- the impact of these findings on HB vaccination procedures, with suggestions as to the best way forward.

While not true of all vaccine-preventable diseases, it has been observed that immunogenicity at primary vaccination is the key predictor for both initial immune response and anamnestic response to HB [5]. It would appear that this is also important for immune memory and, as a consequence, the likelihood of achieving long-lasting protection against the disease. This would suggest that use of a highly immunogenic HB vaccine is desirable to optimize immune memory and provide the best opportunity of achieving long-lasting protection against HB disease.

2. Current status of HB vaccination programmes

Financial constraints have limited the uptake of HB vaccines. Although dramatic decreases in HB vaccine costs in developing countries (e.g. from US$20 to US$0.5–1.00 per person dose) have made mass use of this vaccine in infant immunization programmes possible, it is still more expensive than many other routine childhood vaccinations [3]. At present, periodic booster doses are recommended in many national vaccination schedules, even in immunocompetent individuals, and booster doses are considered for immunocompromised individuals when their levels of antibodies against HBV surface antigen (anti-HBs levels) fall below 10 mIU/ml. The additional costs imposed by the booster campaign have discouraged international agencies from making this vaccine available to the poorer developing countries. However, scientific data do not support the need for routine booster vaccinations to maintain long-term protection in these populations [6]. As a consequence, new recommendations have been put forward, which are summarized in Table 1. These recommendations restrict the need for boosters to immunocompromised ‘at-risk’ groups such as those undergoing haemodialysis, those with chronic renal failure or liver disease, or those who are HIV-positive individuals. The widespread acceptance of this concept would translate into substantial cost savings in both developed and, more importantly, developing nations. However, if booster vaccination is to be accepted as unnecessary, it is essential that consideration be given to optimizing primary vaccination courses.

There is also evidence that it may be possible to reduce the number of doses from three to two in the primary vaccination course, without compromising HB vaccine efficacy [7]. These dose reductions, along with further cost-savings associated with the increasing availability of combination vaccines containing HB, will have a significant impact on accomplishing the global coverage necessary if the goal of HB eradication is to be achieved.

3. What is meant by protection against HBV?

It is important to appreciate the distinction between protection against subclinical and breakthrough infection. Breakthrough infection, which may be identified by the detection of HBsAg and resulting clinical disease, has not been observed following HB vaccination [6]. So called ‘benign’ or subclinical infection results in seroconversion for anti-HBc (antibodies to HB core antigen) occurring with transient viraemia but without symptoms, and without disease. Those individuals who were vaccinated in the past and whose level of anti-HBs decline to low or undetectable levels over time, can mount an anamnestic response within a period as short as 4 days of viral exposure [8]. While infection may be limited to a small number of hepatocytes, rapid antibody production by memory B cells can prevent spread of the virus to larger areas of the liver, hence terminating infection before the person becomes at risk of developing chronic HBV infection [5].

Table 1

<table>
<thead>
<tr>
<th>Groups traditionally considered for booster HB vaccination</th>
<th>Groups considered for booster HB vaccination in new recommendations [6]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunocompetent individuals</td>
<td></td>
</tr>
<tr>
<td>Adolescents (immunized in infancy)</td>
<td></td>
</tr>
<tr>
<td>Adults</td>
<td></td>
</tr>
<tr>
<td>Immunocompromised individuals</td>
<td></td>
</tr>
<tr>
<td>Haemodialysis</td>
<td></td>
</tr>
<tr>
<td>Chronic renal failure/liver disease</td>
<td></td>
</tr>
<tr>
<td>HIV-positive</td>
<td></td>
</tr>
<tr>
<td>Healthcare workers and others at occupational risk</td>
<td></td>
</tr>
<tr>
<td>Travellers</td>
<td></td>
</tr>
<tr>
<td>High-risk groups</td>
<td></td>
</tr>
<tr>
<td>Sexually promiscuous</td>
<td></td>
</tr>
<tr>
<td>Intravenous drug users</td>
<td></td>
</tr>
<tr>
<td>Haemophiliacs</td>
<td></td>
</tr>
<tr>
<td>Mental institution residents</td>
<td></td>
</tr>
</tbody>
</table>
activity from CTLs. In the second study, antibodies were administered to transgenic mice expressing the HBsAg, in which there was a loss of suppression of viral protein gene expression. The results obtained provide the most likely explanation for how so few CTLs are able to clear HBV from so many hepatocytes [14].

5. Hepatitis B vaccine-induced immune response

Much work has gone into characterizing the immune response to the HBsAg, used in recombinant HB vaccine preparations [15,16]. The main recombinant HB vaccines, which have proved so successful since their introduction in 1986, contain yeast-derived HBsAg. Administration of these vaccines leads to HBsAg being presented by a classical pathway, i.e. by antigen-presenting cells (APC) to T helper cells (CD4) that recognize HLA class II molecules on the APC. T helper cells trigger HBsAg-specific B cells to differentiate into anti-HBsAg immunoglobulin (IgG)-producing cells and to proliferate rapidly [17]. Antigen-specific B cells are responsible for the production of anti-HBs (IgG). Following the primary immune response, both memory T cells and memory B cells are generated and, together, they contribute to subsequent vigorous antibody responses [18].

5.1. Relationship between antibody response and lymphoproliferation

The T-cell dependence of humoral immune responses has led investigators to study the relationship between antibody levels and lymphoproliferation. Experiments with spot-enzyme-linked immunosorbent assay (spot-ELISA), an in vitro method of detecting the ability of circulating B cells to produce anti-HBs, have indicated a close correlation between in vitro anti-HBs IgG production and immune memory (indicated by memory B-cell frequency) [19]. A statistically significant correlation has also been reported between the kinetics and magnitude of the in vivo humoral immune response and the in vitro T-cell response. The correlation seen in one selected vaccine recipient from this study of 50 subjects is illustrated in Fig. 1 [16].

It has been hypothesized that T-cell responses reflect B-cell responses. An increasing number of studies report that the highest proliferative T-cell responses occur in subjects displaying the highest in vivo anti-HBs IgG responses [20–22]. Such a strong correlation between the outcome of in vitro T-cell responses and in vivo antibody production provide direct evidence for this hypothesis.
5.2. Immune memory

The mechanistic basis for immune memory is the selective expansion and differentiation of clones of antigen-specific B and T lymphocytes [23]. While the B-cell response is the main factor for immune memory, it is generally accepted that the presence of T cells will enhance B-cell persistence, and that ‘memory’ arises from the complex interplay between memory B cells, memory T helper cells, memory CTLs and antigen/antibody complexes [24]. Immune memory is a key characteristic of specific immune responses and resides in memory B and T lymphocytes sensitized through an initial exposure to antigen [25,26]. These cells remain capable of rapid proliferation, differentiation and, in the case of memory B cells, rapid production of specific antibody upon a subsequent encounter with an identical antigen. Immune memory is therefore seen as an adapted immune response to an antigen that has been previously encountered by the immune system. Under normal conditions, this response involves the production of higher concentrations of serum antibodies after a shorter time than after the first exposure to the antigen, with these antibodies having a higher binding capacity for the antigen [27]. In understanding the factors that influence immune memory to HB vaccine, we must look at the underlying immune mechanisms.

5.3. Persistence of immune memory to HBV

While the CTL response is fundamental to the elimination of HBV from infected hepatocytes, it is the antibody response that halts progression of the infection by neutralizing virus in the blood, mucosa and extracellular spaces. The focus of immune memory against HBV is therefore on the rapid production of protective antibodies. The data suggest the retention of considerable immune memory after loss of detectable anti-HBs Ig [28]. Indeed, anamnestic response, as demonstrated by antibody response following booster vaccination, has been observed in individuals for whom anti-HBs Ig has been undetectable for as long as 28–31 months [5]. Therefore, the decline and eventual loss of antibody following primary vaccination does not indicate loss of immunity, as long as vaccines retain immune memory [7]. Vigorous antiviral T-cell responses have also been revealed several years after clinical recovery from acute HB [29].

Investigation of the persistence of immune memory relies mainly on epidemiological follow-up of vaccinees and booster studies. The status of immune memory can be assessed through the use of the in vitro spot-ELISA [8], and results are consistent with epidemiological and booster data [28]. Vaccine trials and follow-up studies demonstrate the long-term persistence of immune memory to HB over periods of 5–12 years. These studies are summarized in Table 2 [15]. The only study to document cases in which individuals with intact immune memory went on to develop hepatocellular carcinoma involved three Taiwanese civil servants [38,39]. These individuals were HBsAg-negative, but were anti-HBc core antigen (HBc) positive. This suggested a small risk of hepatocellular cancer among those who have anti-HBc as the only marker of a previous infection. However, this study was performed before hepatitis C virus (HCV) was discovered; HCV is now known to be a common cause of hepatocellular cancer. It is suspected that these isolated cases may have arisen as a result of infection with this virus [6]. It has also been suggested that HBV episodes following vaccination are a result of infection with viruses with a surface antigen mutation, referred to as ‘escape mutants’ (reviewed in Ref. [40]). It is possible that these virus mutants may not be recognized by the anti-HBs antibodies raised through vaccination. However, experiments in chimpanzees indicate that immunization with licensed recombinant HB vaccines stimulates anti-HBs that is broadly reactive, and affords protection against infection with the most commonly encountered surface gene mutant of HBV [41].

As the aim of vaccination during infancy and adolescence is to provide long-lasting protection, the vaccine must be of optimal immunogenicity. Studies on the duration of protection against HB disease following vaccination demonstrate that, once a satisfactory level of immunity to HB has been induced, it persists, even when antibody levels become undetectable. This suggestion that immunological priming occurs in adequately vaccinated individuals is supported by studies in which HBsAg-specific memory lymphocytes are retained in the peripheral circulation of vaccinees, even if they are no longer producing detectable levels of antibody. However, it has been observed that the antibody response to a full primary vaccination series is closely correlated with the expected response to re-vaccination. The immunogenicity of the primary vaccination is therefore a key factor in determining the strength of the subsequent anamnestic response (Fig. 2) [5]. As anamnestic antibody response is dependent upon memory B cells, it is reasonable to interpret the higher anamnestic response as being indicative of a stronger B-cell memory capacity, reflecting the immunogenicity of the primary vaccination schedule.

Studies in which immune memory has been assessed through booster vaccination not only confirm the presence of immune memory [7,15,36,37,42], but also support the recent recommendation that the booster is not actually needed to sustain protection [6]. Consequently, there is a substantial body of experimental evidence that lymphoproliferation is highest among subjects with the highest in vitro anti-HBs Ig responses following primary vaccination. This is supported by clinical ob-
### Table 2
Hepatitis B infections in immunized populations

<table>
<thead>
<tr>
<th>Population</th>
<th>Vaccine and schedule</th>
<th>Number of subjects</th>
<th>Time since vaccine (years)</th>
<th>% retaining ≥10 mIU/ml anti-HBs</th>
<th>Number of HBV episodes</th>
<th>Clinical manifestation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alaskan natives [30]</td>
<td>P at 0, 1, 6 months</td>
<td>10,37–1497</td>
<td>7</td>
<td>74</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Hong Kong children [31]</td>
<td>R at 0, 1 month (5 µg)</td>
<td>72–100</td>
<td>5</td>
<td>75</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>R at 0, 1, 6 months (5 µg)</td>
<td>63–101</td>
<td>5</td>
<td>87</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>P at 0, 1, 6 months (10 µg)</td>
<td>64–105</td>
<td>5</td>
<td>84</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Senegalese infants [32]</td>
<td>P at 0, 1, 2, 12 months</td>
<td>92</td>
<td>9–12</td>
<td>88 (protective efficacy)</td>
<td>18</td>
<td>2</td>
</tr>
<tr>
<td>Taiwanese neonates of carriers [33]</td>
<td>HBIG at birth; R at 0, 1, 6 or 0, 1, 2, 12 months</td>
<td>140</td>
<td>5</td>
<td>83</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>US neonates of carriers [34]</td>
<td>HBIG at birth; P at 0, 1, 6 months or R at 0, 1, 6 months</td>
<td>70</td>
<td>4–9</td>
<td>87</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Belgian adults [35]</td>
<td>R at 0, 1, 2, 12 months (20 µg)</td>
<td>40</td>
<td>8</td>
<td>93</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>New Zealand children [36]</td>
<td>P at three-dose schedule (2 µg)</td>
<td>125</td>
<td>9</td>
<td>95</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>Italian infants [37]</td>
<td>R at 1, 2, 3 or 3, 5, 11 or 3, 5 months</td>
<td>168</td>
<td>10</td>
<td>60, 66, 56</td>
<td>0, 0, 1</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>R at 1, 2, 3; or 1, 3; or 3, 5, 11; or 3, 5, 5 months</td>
<td>419</td>
<td>5</td>
<td>80, 33, 97, 91</td>
<td>0, 0, 2</td>
<td>0, 0</td>
</tr>
<tr>
<td>US healthcare personnel [38]</td>
<td>P administered by a 0, 1, 6 month or 0, 1, 2, 14 month schedule</td>
<td>985</td>
<td>6</td>
<td>85</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

*Surveillance applied to all vaccinees, 95% of whom initially developed ≥10 mIU/ml anti-HBs. Three of the eight breakthrough infections detected involved subjects with an initial response <10 mIU/ml. P, Plasma-derived vaccine; R, recombinant; anti-HBc, antibodies to hepatitis B core antigen; HBIG, hepatitis B immunoglobulin; NA, not available. (Updated after Ref. [16].)
servations that the higher the antibody response to the primary vaccination course, the higher the expected response to revaccination.

6. Role of antigen in B-cell and T-cell immune memory

There have been a number of more general studies on the underlying mechanisms determining immune memory that allow us to gain a better insight into the situation with HB specifically, and these are now presented.

6.1. Antigen dependence

Stimulated antibody-dependent immune memory, requires increased and persistent levels of neutralizing antibodies. Without persisting antigen, elevated antibody levels have been reported to be short-lived [43]. B cells stimulated by antigen–antibody complexes, captured on follicular dendritic cells in germinal centres for prolonged periods of time, are thought to be the basis for the production of protective antibodies following exposure to virus [44]. However, the existence of a more antigen-independent type of memory B cell was reported as early as 1990 [45], and it has been shown recently that antibody-producing plasma B cells are more long-lived in the absence of persisting antigen than was previously assumed. These long-lived plasma cells contribute to B-cell memory [46]. It would appear, therefore, that there are two distinct sets of memory B cells: one population of recirculating B cells that do not proliferate, and a second population of memory B cells that remain associated with persisting antigen, proliferate and differentiate to antibody-forming cells. This is supported by the studies of Bachmann et al. [44], who used a vesicular stomatitis virus (VSV) model system to analyse the localization of virus-specific B cells at different time points after immunization. They observed that, whereas the maintenance of antibody titres depend upon the presence of persisting antigen, resting memory B cells appear to be less antigen dependent. They also suggested that only memory B cells activated by persisting antigen differentiate to antibody-forming cells, and produce and maintain protective antibody levels [44]. The relative importance of each of these B-cell memory populations in the persistence of antiviral protection remains to be established.

Although the characteristics of cytotoxic T-cell memory parallel the characteristics of B-cell memory, the question of whether CTL cell memory generally depends on persisting antigen has been the subject of a number of studies [25,47–49]. Such studies have shown that the maintenance of increased CTL precursor frequencies and T-cell memory residing in lymphoid tissues is long-lived, antigen independent and protective against systemic, blood-borne infections. In contrast, for protection against peripheral viral infections occurring outside the lymphatic and vascular system, in the absence of antigen, T-cell memory is short-lived. The antigen-dependent CTL memory population may therefore represent an effector population stimulated by a low-level persisting virus and may reflect an ongoing low-level T-cell response that keeps non-cytopathic or poorly cytopathic viruses such as HB under control. The discrepancy in the protective capacity of the two memory populations probably lies in their different homing properties. Continuously induced and activated effector memory T cells are attracted more rapidly to the site of infection than resting long-lived memory T cells [25,50,51] and may thereby interfere quickly and efficiently with virus spread. Conversely, long-lived resting memory CTLs are reactivated more efficiently than naïve T cells and are present at higher frequencies. They can therefore promote a limited but sufficient degree of protection, particularly if the antigen reaches the lymphoid system. By analogy, antibody-mediated protective immunity may not only be promoted by elevated neutralizing antibody titres maintained by continuous antibody production, but may also be preserved by elevated levels of rapidly reactivated long-lived antigen-independent resting memory B cells that enhance the elimination kinetics of the pathogen. Such a view could explain the phenomenon where,

Fig. 2. Correlation between primary vaccination and booster vaccination. Reproduced with permission from Jilg, 1990 [5]. Note that the antibody response to a primary vaccination series is closely correlated with the response to re-vaccination.

2 VSV and recombinant HBV vaccine show structural similarities, in that they both have a rigid and highly organized virus envelope, and control of re-infection of both VSV and HBV infection is mediated by antibodies.
although vaccinees with undetectable anti-HBs antibody levels could become transiently infected, the vast majority of such cases showed no signs of hepatitis-associated disease [5].

6.2. Antigen dose

While the exact role of antigen–antibody complexes (persisting antigen) in maintaining a population of memory B cells is not yet defined, there is clearly a role, and it is likely to be aided by the presence of higher antigen content. Transfer of memory cells to antigen-free recipients demonstrated a rapid decline in frequency of these cells during the first few weeks, and less rapid decay thereafter, similar to antibody decay [27]. Later studies have shown a gradual decline in memory B-cell frequency with constant kinetics in the absence of antigen complexes [52,53]. In a study investigating factors influencing the level of neutralizing anti-VSV IgG in a mouse model system, Bachmann et al. identified the amount of persisting antigen–antibody complexes as being the major influence, rather than initially available T helper and B cells [54]. These findings support the opinion that the efficiency of vaccines in inducing long-lasting protective IgG is regulated predominantly by the amount of persisting antigen–antibody complexes, which is controlled by the antigen dose administered.

Similarly, Kundig et al. have shown that the duration of T-cell memory protective against peripheral re-infection depended on the antigen dose and form used for immunization. They reported that protective immunity correlates either with the duration of antigen persistence or with the extent of clonal expansion of T cells (clonal burst size) during the early immune response [50]. Thus, higher initial antigen doses translate into longer antigen persistence and a more vigorous initial T-cell response.

As a general concept, it appears that the antigen dose, its distribution and the duration of antigen exposure to immune cells in secondary lymphoid organs is an important factor in determining the immunogenicity of an antigen [55].

6.3. Nature of the antigen

The nature of the antigen is also a critical factor for the initiation of a potent immune response and, thus, for the establishment of immune memory. This is particularly important in vaccination, where it is generally considered that live vaccine leads to a long-lasting response, whereas inactivated vaccines and protein vaccines induce more short-lived memory [56]. However, recent studies have shown that highly repetitive antigens, such as the HBsAg of non-infectious particles used for the HB subunit vaccine, produce strong T-cell-independent B-cell responses [57,58]. Furthermore, the initial immune response is key in determining the duration of immunity for non-replicating antigens.

These studies provide more detailed information on the influence of the antigen presented to the immune system on the subsequent development of immune memory (Fig. 3), demonstrating that:

- antigen persistence is important in the generation of antibody-forming memory B cells and, hence, maintenance of protective antibody levels;
- antigen persistence is dependent on the antigen dose administered, increasing with increasing antigen dose;
7. The way forward

Further elucidation of immune memory will undoubtedly affect future vaccination policies for HB. Studies into the threshold level at which B cells provide lasting memory and on the synergy between T- and B-cell responses would assist our understanding of the development of long-lasting immune memory. Long-term follow-up studies should continue to monitor groups of individuals immunized against HB, investigating both humoral and cellular immunity in these groups. The effect of combining HB vaccine with other childhood vaccines should also be assessed, as the move to use of combination vaccines is an important goal in the fight against childhood diseases.

Current evidence for the role of the primary immune response in the persistence of immune memory makes it important that an optimal immune response to primary vaccination is guaranteed. This is even more important in view of the desirability of reducing the number of doses of vaccine administered.

8. Summary

This review presents scientific evidence that gives us an insight into the question of lifelong immunity to HB disease through vaccination. The main findings can be summarized as follows. Immune memory arises from a complex interplay between memory B cells, memory T helper cells, memory CTLs and antigen–antibody complexes. Immune response to disease and to vaccine is B- and T-cell mediated. There is a link between lymphoproliferation (T-cell activity) and in vivo humoral response, as well as between in vitro IgG anti-HBs production and immune memory (memory B-cell activity). Immune memory can persist even after loss of detectable antibodies. However, the strength of the anamnestic response is related to the antibody response to the primary vaccination course; thus, the duration of immune memory would appear to be correlated to the antibody response. There is a link between antigen dose and level of neutralizing antibody in that the vaccine antigen dose controls the amount of persisting antigen–antibody complexes, regulating the level of neutralizing antibodies. Higher vaccine antigen dose is related to longer antigen persistence and, subsequently, a more vigorous initial T-cell response. Antigen persistence, dose and nature determines the level of antibodies and the T-cell response, and consequently influences the strength of the anamnestic response, lymphoproliferation and the B- and T-cell-mediated immune memory.

References


