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ON IMMUNITY AGAINST INFECTIONS

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У роботі розглянуто різні ланки імунітету, представлено альтернативні підходи до трактування поняття «імунологічна пам'ять», описано неоціненну роль вакцин і обгрунтовано сучасні вимоги, що ставляться до щойно створюваних вакцин.
Ключові слова: імунітет, вакцини, інфекційні хвороби, чутливість.

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В работе рассмотрены различные звенья иммунитета, представлены альтернативные подходы к трактовке понятия «иммунологическая память», описана неоценимая роль вакцин и обоснованы современные требования, предъявляемые ко вновь создаваемым вакцинам.
Ключевые слова: иммунитет, вакцины, инфекционные болезни, чувствительность.

Introduction

During the past 100 years the nature of immunological memory has been widely debated, not only by basic immunologists but also in the clinical context and from a social and preventive, medical point of view[1–4]. Vaccinations against classical childhood diseases, including polio, measles etc., have been very successful, and poxvirus has been successfully eradicated by general vaccinations with vaccinia virus. Nevertheless, efficient vaccines are still lacking against tuberculosis, leprosy and most classical parasitic diseases, including malaria, leishmaniasis and shistosomiasis. Importantly, vaccines are also lacking against HIV, herpes simplex virus type I and II, against papilloma virus infections or against most tumors. In addition, some vaccines, including measles and mumps, are less efficient than others since re-infections rarely may "break through".

Immunological memory

In immunological textbooks immunological memory is usually explained as a special quality of T or B cells that have acquired a new status of "memory" when compared to naïve cells or effector cells. This status, which lies beyond increased precursor frequencies, enables the system to respond more quickly and therefore more efficiently to a second exposure of a particular antigen or an infectious agent[5–8]. The nature and the special qualities of this "memory status" are however still incompletely understood. The alternative view is that immunological memory is basically a low-level antigen-driven immune response, consequently protection by immunological memory eventually disappears without antigen. Of course, these two views differ drastically and have important consequences as to how vaccines function over time against infectious agents (or tumors). Therefore it should be important to distinguish between these two alternatives.

On serotypes and the importance of neutralizing B cell responses versus helper or cytotoxic T cell responses

The highly repetitive paracrystalline identical determinants on most infectious agents induce T-independent B cells responses very efficiently by maximal cross-linking of immunoglobuline receptors on B cells. The importance of these highly efficient IgM B cell responses is to rapidly expand neutralizing B cells during an early phase of the infection. The many B cells offer sufficient targets for T help that has in the meantime been induced about by day 4 or 6 after infection via antigen-presenting cells. They are therefore readily switched to IgG-producing plasma cells. This switch is very important because IgM, due to its size, cannot diffuse readily into solid tissues; in particular, only IgG may reach the central nervous

system. In fact, the initial precursor frequency of neutralizing antibody producing B cells against viruses is low in the order of 10^{-4} to 10^{-5}. This low frequency contrasts with the 10 to 100 times higher frequency against viral internal antigens or the 100–1000 times greater initial frequency of B cells specific for chemically defined small haptens[9;10].

Both cytotoxic and helper T cells against serotype-defined virus groups are virtually completely shared between the various serotypes. The well recognized lack of cross-protection between serotypes (e.g. poliovirus I, I, III) under epidemiological conditions clearly indicates that neutralizing antibodies and not primed helper or cytotoxic T cells are limiting protection[11]. Importantly, in the examples of polio or influenza viruses, no cross-protection by primed T help or cytotoxic T cell responses is observed in primed hosts despite the increased precursor frequencies of memory T helper and memory cytotoxic T cells. This does not really come as a surprise except that serotypes have been much forgotten during the past 20 or 30 years because of over-emphasis of T cell-mediated immune effector mechanisms. In fact, reliable epidemiological evidence teaches that sufficient neutralizing titers against acute infectious agents correlate with efficient levels of protection. Of course, this does not mean that non-neutralizing antibodies are not useful parameters to monitor the infection history of a patient; they are more easily measured by ELISA. Also, because neutralizing titers often correlate with ELISA titers (but not always, e.g. against non- or poorly cytopathic infections such as HBV, HCV, HIV, LCMV, TB) one gets away with this inadequate measure.

While most would agree that sufficient neutralizing antibody titers are a reliable measure for protection against infection with cytopathic agents, such serological parameters are not readily available for measuring protection against non-cytopathic persistent agents where T cell immunity is also of key importance. However, as stated earlier, because these agents are poorly or non-cytopathic, evolutionary pressure is low, except for the paradoxical pressures exerted by unbalanced immune T cell responses resulting in immunopathology[12–14].

Why should immunological memory be necessary?

Immunological memory describes the fact that humans infected once with measles-, pox- or polio viruses are subsequently resistant against disease caused by re-infections. While immunological memory has been a subject of many years of immunological inquiry and experimentation, it has been only rarely analyzed from an evolutionary point of view. Cytopathic infections usually kill immunological low responders rapidly whereas high responders tend to survive. Immunological memory cannot improve these overall conditions in general, except if vaccines had been foreseen by evolution, but this is of course not the case, at least as we understand conventional vaccines today. Nevertheless, prevention of infection or attenuation of infection during critical embryonal and fetal phases and after birth is the co-evolutionary key to understand immunity and immunological memory[15;16]. During these periods of physiological immuno-incompetence "immunological memory" is essential for the survival of the species as will be pointed out below. Here it suffices to "over"-state the case that, if a naive host survives a first infection, this host basically does not need immunological memory to survive the second infection. Vice versa, if a host does not survive the first infection, he certainly does not need immunological memory thereafter.

Immunological memory by neutralizing antibodies. A crucial question therefore is: during which period during a higher vertebrate's life is the host immunologically unprotected against infections and how can protection be provided to the host from the outside during this critical period? As pointed out above, during the phase before and right after birth, there is an absolute need for passively acquired immunoprotection, because offspring are immuno-incompetent. This may be best explained as a consequence of MHC-restriction of T cell recognition requiring MHC-polymorphism. To avoid easy selection of viral mutants evading MHC-restricted T cell recognition, MHC-polymorphism developed during phylogeny and endangered maternal fetal relationships in ontogeny. This inherent danger of graft versus host- or host versus graft reactions between mother and offspring is avoided by lack of MHC antigen expression in the placental contact areas, by general immunosuppression of the mother, and by virtually complete immunodeficiency of offspring up till birth. Immunological experience that can be adoptively transferred from mother to offspring therefore is an absolute requirement to overcome infectious diseases in utero and during the first few weeks and months after birth, a period needed for full maturation of the new-born's immune system. Therefore, protective antibodies that exist at sufficient levels in the serum of the mother are passively transmissible soluble forms of an immunological antibody-memory, essential for the protection of offspring during the period needed for the development of T cell competence and the capacity of the offspring to generate T help-dependent IgG protective neutralizing antibody responses. This result of co-evolution would have rendered highly unlikely development of cytopathic agents that could not be controlled sufficiently by adoptively transferred antibodies during this critical time. From this point of view one may postulate that without already existent — or in parallel de-

veloping — soluble transmissible immune protection by antibodies, both MHC-polymorphism and MHC-restricted T cell recognition could not have developed phylogenetically[4;16].

Impressive examples illustrating the great importance of adoptively transferable immunological experience by antibodies from mother to the immuno-incompetent offspring are agamma-globulinimia or severe combined immunodeficiency in humans, in mice or in newborn calves. Infants, incapable of generating their own immunoglobulins, will be protected by maternal antibodies for the first 3–6 months after birth, because antibody is transferred via placenta (but not via milk to serum but obviously human milk antibodies are active within the gut). In contrast to humans and mice, calves are born without serum immunoglobulins, because maternal immunoglobulin cannot pass through the completely double-layered placenta. Calves can and must take up via gut colostral maternal immunoglobulins during the first 18 hours after birth. During this short time period, gut epithelia transport immunoglobulin to the blood. If this does not happen, calves will remain without maternal protective antibody and die of various infections during the next few weeks, because their own yet immature immune system cannot act quickly enough to mount effective protective immune responses[15].

The key question then is: how can sufficient protective antibody levels be maintained in serum and milk of mothers to provide protection for offspring? Protective antibody levels cannot be generated during the 270 days of a human or the 20 days of a mouse-pregnancy respectively to cover all relevant infectious diseases threatening survival of the embryo and of the newborn. In fact, infections during pregnancy must be avoided. Many acute cytopathic but also poorly cytopathic infections during pregnancy will cause abortion or developmental abnormalities. Therefore, by co-evolutionary necessity, all life-threatening acute infections must be survived by mothers before puberty. Therefore, the so-called childhood diseases represent the co-evolutionarily-balanced infectious disease experience before procreation, and immunological memory represents accumulated immunological experience and protection before pregnancy. This proposal includes additional benefits of an improved immune system during adult live, including herd-immunity, as will be pointed out below; nevertheless, one can only die once in real life, and that is during the early period after birth. The important aspect of immunological memory transmissible from mother to offspring is further indicated by an important hormonal regulation of antibody responses in that oestrogens and progesterones improve overall antibody-responsiveness in females compared to males. This, as a consequence, correlates with the 5:1 higher ratio of autoantibody-dependent autoimmune diseases suffered by females compared to males.

Of course the differences between males and females in maintaining memory antibody titers are relative and indicate additional, though less early and less directly life-limiting roles of immunological memory: it not only increases individual fitness, but importantly contributes crucially to herd-immunity. Herd-immunity describes the equilibrium at the population and species level between susceptible and immune individuals; it depends on the infectious agent (acute or persistent) but also on the level of immunity (neutralizing antibody titers or/and activated T cells) and on the population density but also on animal reservoirs (e.g. other vertebrates, but also insects).

On cellular immunity

What then is the role of cell-mediated immune protection and of protective T cell mediated memory? Many experiments in mice have demonstrated that adoptively transferred CD8+ T cells protect against acute infections with non-cytopathic viruses. Under very defined conditions such experiments have also been done successfully against cytopathic viruses including influenza virus. But as stated earlier, the fact is that humans or mice are not efficiently protected against distinct serotypes of viruses despite shared CD8+ and CD4+ T cell specificities. This strongly indicates that such T cell responses can not cross-protect. If T cells are however acutely activated, they can exhibit a protective phenotype during the period of activation; for CD8+ T cells, specific for acute cytopathic virus such as rhabdo or influenza virus, this period lasts only for about 3 weeks. Experience with many infectious diseases, including tuberculosis, leprosy or HIV nevertheless demonstrates that T cell memory provides efficient protection against re-infection. Importantly however, this protection is relevant for the host and the population (and not directly for the offspring). The infections controlled crucially by T cells are largely non-cytopathic and slow in nature with a tendency to persist. These infections will not kill the host rapidly but rather tend to establish a balanced state of infection-immunity, i.e. survival of low numbers of infectious agents, usually in granulomata within the host, balanced and controlled by an active ongoing T cell-mediated immune response[13;17;18]. Thus, T cell-mediated immune protection and T cell memory are directly relevant for the infected host. There is a third interesting and revealing category of infectious agents besides the acutely cytopathic ones or the poorly or noncytopathic persistent infections, this third group comprises the variably pathogenic ones exemplified best by herpes viruses. Herpes viruses and alike induce both humoral and cellular immunity. Because they are variably causing limited i.e. usually non-life-threat-

ening infections, their immunological handling leaves a lot of leeway for the infection to get through and establish normally local non-life-threatening and limited persistent infections.

Non-cytopathic agents such as HBV, HCV or possibly HIV are transmitted before or at birth via maternal blood[16]. Because the offspring are immuno-incompetent and because obviously maternal immune defenses against these agents have failed, virus are best transmitted during this period of time without endangering survival neither of offspring nor of the host species. Some of the persistent non-cytopathic infections may eventually cause serious disease in the host, such as primary liver carcinomas by HBV infections or perhaps some chronic autoimmune diseases or chronic immunopathologies, but these consequences of chronic persistent infections usually show up much later than needed for the species to procreate and survive.

Maintenance of immunological memory

As pointed out, sufficiently high neutralizing protecting antibody titers primarily in mothers but for herd-immunity also in sufficient individuals are essential to guarantee survival of offspring and of the species. Such maintenance of high neutralizing antibody titers may be achieved via:

1) Re-exposure to the antigen from external sources, e.g. poliovirus infections. Nowadays re-exposure is via the Sabin vaccines spread within the household or via public swimming pools, in former times via periodic subclinical reinfections starting early in life under the umbrella of maternal antibodies (see below).

2) Re-exposure from antigen sources within the host. This mechanism is probably a key to understand measles virus immunity. Measles virus persists in the host not as replication-competent virus but as crippled virus usually missing a functional matrix protein. The fact that about 1:1000 to 1:3000 wild type measles-infected children develop subsclerosing panencephalitis (SSPE) is just the tip of the iceberg. This disease correlates with persistence of virus in central nervous tissue. This persisting measles virus is crippled because the matrix protein is defective by mutation. Similarly, HBV virus in men or LCMV virus in mice[19] persists at very low levels and boosts immune responses of T and B cells repeatedly.

3) Antibody-antigen complexes on follicular dendritic cells are maintained for long periods of time and boost both antigen-specific B cells as well as T helper cells. Since cross-priming and cross-processing of inert antigens can only exceptionally access the MHC-class I in APC these antigen depots are in general neither capable of maintaining CD8 T cell memory, but importantly are also not reduced or eliminated by CTLs[20].

In absence of antigen-boosts antibody responses will eventually dwindle, this includes neutralizing antibody responses against tetanus toxin or diphtheria toxin or polio vaccines; this clearly indicates that long-lived plasma cells alone cannot be responsible for maintenance of protective antibody memory.

Vaccines

Vaccines represent the greatest successes of medicine. Interestingly, all working vaccines protect hosts via neutralizing antibodies. This includes the classical childhood vaccines against bacterial toxins, measles, polio and pox. The important aspect here is that vaccines in general do not prevent re-infection but reduce infections so as to prevent disease. Thus sterile protection is probably not or very rarely achieved with any of these vaccines. Overall, these clinical experiences illustrate first that T cell-mediated memory is short-lived and depends on ongoing infection, and second, that neutralizing antibody memory preventing hematogenic spread of the same virus is long-lived and is maintained for long time periods by any of the mechanisms discussed above.

Protective vaccines

All vaccines that work and provide proven protection both for the individual as well as the population are vaccines that induce at least neutralizing antibody responses of relatively long duration[3;21]. Vaccines that do not work include long-term protection by vaccinations against tuberculosis, leprosy, most classical parasite infections, but also against some viral infections, including herpes papilloma and HI virus. All these infections have in common that neutralizing antibodies alone are not sufficient to eliminate or control the virus. But as pointed out above, these infections are by themselves either poorly or non-cytopathic (HIV, leprosy, etc.) or usually not efficiently lethal for the host species (e.g. herpes viruses). All these agents need both antibodies and T cell mediated effector-mechanisms for their efficient control, and importantly, this T cell mediated protection usually depends also on constantly activated effector T cells to control further spread and expansion of the infection. While CD4 T cell memory may be maintained by inert non-replicating antigen that may be stored as immune complexes on follicular dendritic cells or in granulomas (if the antigen is poorly digestible and/or mixed with lipids), cytotoxic protective T cell memory is dependent on persistent infection. As stated above, the reason simply is that the class I-pathway of peptide loading generally depends on intracellular generation of peptides. The few experimental exceptions to this rule cannot reverse this fact. In a few rare exceptions class I MHC-loading may also be achieved from the outside of a cell, particularly in vitro. This has been shown convincingly for great amounts of ovalbumin in model situations in mice.

Cytotoxic T cells have the key function of controlling non-cytopathic virus causing extra lymphatic infections in peripheral solid organs. Although protective during the period of acute infection, they may also be detrimental because of immuno-pathological destruction or otherwise innocuously infected host cells. Therefore, excessive cytotoxic T cell responses against too many non-lytically infected target cells of the host causes disease and therefore should be avoided. This delicate balance between immunoprotection and immunopathology is well illustrated in the various phenotypes of HBV infection-caused disease; in apparent (low virus, very efficient immune response) or apparent infection with rapid recovery within a few weeks, acute or chronic, aggressive hepatitis (high virus and variable, quick or slow T cell responses) caused by immunopathology in about 1 to 2 percent of HBV-infected patients and establishment of a clinically in apparent virus carrier state in very few (much virus, little or no T cell response). As stated above, in contrast to serum antibodies, primed cytotoxic T cells cannot be transmitted to offspring because of the usual transplantation antigen differences between mother and offspring. Therefore, primed cytotoxic T cell responses primarily function to prevent virus spread within the same host; an efficient early response limits or prevents immunopathological disease. If virus is controlled down to low levels, no chronic disease develops, or only late. If, however, virus has spread — or spreads again — widely, severe auto-aggressive disease may develop. A similar balance exists in leprosy or TB infections. In all these examples nobody would argue against the obvious fact that low-level infection maintains protective immunity. Mackaness coined the term "infection-immunity" or "infectious immunity" to describe this important co-evolutionary equilibrium. Interestingly, most of these chronic infection-immunity states are accompanied by a heightened degree of macrophage-activation via interleukins (e.g. interferong and TNF) and probably activate natural killer cells. Such an increased activation status enhances basic non-specific initial handling of low-level infections. Such a state of concomitant non-specific infection-immunity is not only beneficial to control the specific infection but may also contribute considerably to improved basic or "natural" host resistance. Therefore these kinds of chronic low level infections may represent an exquisite evolutionary balance of mutual benefit between vertebrate hosts and infectious agents.

From all this we conclude that immunological memory represents not necessarily special characteristics of lymphocytes but rather reflects co-evolutionary balances, where low-level responses driven by antigen that is either stored as immune complexes on follicular dendritic cells or is re-encountered from persistent localized infections, such as granulomas or few infected cells in peripheral solid organs (CNS, kidney, lung) or from infections from the outside. While antibody memory is of key-importance to transfer protection to the immuno-incompetent offspring, T cell memory (often combined with antibodies) is important to control persistent infections within the individual host. Those vaccines that imitate this co-evolutionary situation of acute cytopathic agents and induce neutralizing antibodies have been very successful so far. Those vaccines that aim at providing T cell-mediated memory and protection have not been satisfactory because these vaccines have not been able to imitate the key-characteristics of infection-immunity; they are usually not persistent at clinically in apparent low levels of infection within hosts to constantly activate protective effector T cells.

Vaccines that we do not have

Therefore, what we need is a long-term active vaccine that persists without causing disease against TB or leprosy, or that can provide cell-mediated protection against HIV or HCV. Vaccines should guarantee periodical or constant generation of MHC class I-presented peptides in secondary lymphoid tissues to activate CD8+ T cells. Attempts to achieve this with so-called attenuated vaccine strains have either only offered time-limited protection, such as BCG for a few years in small children, or has not been successful so far for leprosy, HCV or HIV infections.

Conclusion

Protection generated by vaccines is a great success of medicine. Vaccinations have prevented more deaths than possibly any other active medical measure taken so far. Because immunological memory is a result of a highly equilibrated co-evolution of infectious agents and the vertebrate immune system, immune protection and successful vaccines cannot be regarded in splendid isolation of academic immunology. Immunity is about protection against infection within an evolutionary context. This is particularly important during the early phases of life, because the immune system of higher vertebrates is immature due to MHC-restricted T cell recognition. Successful vaccines are those that can imitate generation of neutralizing antibody responses against acutely cytopathic agents against which they seem to be the only limiting factor. In contrast, cell mediated immunity against infections that persist in the host are much more difficult to imitate, because the balance between attenuation on one side and, on the other side, persistence versus persistence of the infection to provide constant stimulation of protective effector T cell responses has not been achieved so far to a level of perfection, achieved by million years of co-evolution of host and infectious agents

that tend to persist. Similar problems are exhibited by classical parasites, which in their co-evolution over time have come to innumerable sophisticated host-infectious agents balances that will be considerably more difficult to imitate or beat even compared to TB, leprosy or poorly cytopathic viruses, such as HIV. But the aim should be to develop strategies that aim at exactly that perfection of low-level persisting infections, exemplified by TB, HIV, HCV, HBV and of most classical parasitic infections. While, this may not be easy, development of DNA-types of vaccines hopefully may bring us closer to such a goal.

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