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ON NATURAL AND ARTIFICIAL VACCINATIONS

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■ **Abstract** This review summarizes the general parameters of cell- and antibody-mediated immune protection and the basic mechanisms responsible for what we call immunological memory. From this basis, the various successes and difficulties of vaccines are evaluated with respect to the role of antigen in maintaining protective immunity. Based on the fact that in humans during the first 12–48 months maternal antibodies from milk and serum protect against classical acute childhood and other infections, the concept is developed that maternal antibodies attenuate most infections of babies and infants and turn them into effective vaccines. If this "natural vaccination" under passive protective conditions does not occur, acute childhood diseases may be severe, unless infants are actively vaccinated with conventional vaccines early enough, i.e., in synchronization with the immune system's maturation. Although vaccines are available against the classical childhood diseases, they are not available for many seemingly milder childhood infections such as gastrointestinal and respiratory infections; these may eventually trigger immunopathological diseases. These changing balances between humans and infections caused by changes in nursing habits but also in hygiene levels may well be involved in changing disease patterns including increased frequencies of certain autoimmune and degenerative diseases.

INTRODUCTION

During the past 100 years the nature of immunological memory has been widely debated, not only by immunologists but also in the clinical context and from a public health perspective (1–7, 7a). Immunological memory is the basis for protective vaccines. Vaccinations against childhood diseases, such as poliomyelitis and smallpox, have been very successful, and smallpox has been eradicated by a worldwide campaign with the vaccinia virus (reviewed in 8–12). Nevertheless, efficient vaccines are still lacking against tuberculosis (TB), leprosy, and parasitic diseases, such as malaria, leishmaniasis, and schistosomiasis. Vaccines are also lacking against human immunodeficiency virus (HIV), dengue, respiratory syncytial virus (RSV), Epstein-Barr virus (EBV), cytomegalovirus (CMV), rotaviruses, herpes simplex virus (HSV), and papillomavirus infections and against most tumors. In addition, some antiviral vaccines, such as those against measles

and mumps, are far from offering complete protection since viral breakthroughs may occur (13, 14). These successes and failures or inadequacies demonstrate that our understanding of the nature of immunological memory is incomplete. This review considers the following questions: Have artificial vaccines been "foreseen" by nature? What is the physiological equivalent of our vaccines? What is missing in our knowledge not only about immunity but also about vaccines?

MECHANISMS OF IMMUNE PROTECTION

Resistance to infections is based importantly on nonspecific mechanisms (interferons, complement, natural antibodies, natural killer cells, activated phagocytes) and many additional mechanisms (10, 15–17). These nonspecific resistance mechanisms are responsible for the major part (>95%) of host defense. For example, absence of interferon receptors increases susceptibility of mice to viral infections by several orders of magnitude (18). Specific immunity is phylogenetically a rather new fine-tuner of resistance, emerging as a result of coevolution between hosts and infectious agents.

The two arms of the immune system, humoral and cellular, fulfill the following major tasks. The immunoglobulin receptor of B cells and secreted antibodies directly recognize complex folded proteins or carbohydrates. Protective antibodies inactivate and block the action of infectious agents or toxins by covering them and/or by facilitating their phagocytosis. Immunoglobulin M (IgM) and IgG protect against antigens in blood and the lymphatic system, IgA protects on mucosal membranes (19, 20), and IgE triggers mast cells and basophils in skin and mucosae. In contrast to B cells and antibodies, T cells recognize small peptides presented on the cell surface by major histocompatibility complex (MHC) antigens (21). Cytotoxic $CD8^+$ T cells are specific for fragments of proteins synthesized by the cell itself and are presented by MHC class I (HLA-A, -B, -C) antigens; this pathway includes not only self-peptides but also viral, intracellular bacterial, and tumor antigens. Phagocytized antigens are processed in phagolysosomes and are presented by MHC class II antigens. Dendritic cells, which are either infected themselves or are able to take up infectious foreign antigen or decaying self-antigen, transport antigens to organized lymphatic tissues. They are therefore often of key importance in inducing T cell responses. Whereas antibodies act directly where they are released or transported to, T cells actively emigrate into peripheral solid tissues. T cells can act via direct contact or by specific release of immune mediators such as interferon or tumor necrosis factors (TNFs), or they can act nonspecifically via recruitment and activation of macrophages. Cytopathic viruses or bacteria that cause an acute lethal infection are in general most efficiently controlled by soluble diffusible factors including T cell–dependent cytokines [such as gamma interferon (IFNγ) and TNF] and by specific neutralizing antibodies. Noncytopathic intracellular organisms usually cause no direct cell or tissue damage and therefore no disease, even though they tend to persist. In this case immune control is mediated by perforin-dependent, cytotoxic, and cytokine-releasing T cells that

TABLE 1 General rules for induction and maintenance of effector cells and antibody responses

T cells
Ignore antigen not reaching lymph nodes, spleen, or Peyer's patches in sufficient dose and for less than 3 days.
Some T cells are induced against antigen reaching lymph nodes, Peyer's patches, and/or spleen in moderate doses and dose kinetics for 3–15 days.
All are induced and exhausted if antigen persists at sufficient levels everywhere. (This is negative selection, which occurs earliest in thymus, but also in secondary lymphatic organs, lymph nodes, and the spleen.)
Maintenance of immediately effective cytotoxic or helper T cells depends on presence of antigen.
B cells
Induced only in secondary lymphatic organs (lymph nodes, spleen, Peyer's patches, crypto patches).
Induced by rigid, multimeric, highly concentrated antigenic determinants or multimeric antigens together with LPS-like polyclonal activators: IgM responses of T independent type 1.
Induced by multimeric, mobile, or flexible antigens (on cell surfaces or linear flexible multimers) together with unlinked T help: IgM responses of T-independent type 2.
Induced by limiting doses of mono- or oligomeric antigens to make IgM or IgG responses if conventionally linked T helper cell activity is provided: T help–dependent B cell responses.
Switched to IgG-dependent upon carrier-specific conventionally linked T help.
Maintenance of antibody titers in serum depends on antigen-driven B cell maturation to plasma cells.

cause inflammation and tissue damage (22, 23). Since the immune system cannot distinguish a priori between cytopathic and noncytopathic infections it cannot really "foresee" its beneficial and detrimental effects on the host; it merely responds to antigen. Therefore, protection by immunity represents an equilibrium between optimal resistance against the various cytopathic infections and avoidance of excessive immunologically mediated tissue damage. Clinical examples of unbalanced immunity against non- or weakly cytopathic infections causing disease by immunopathology are tuberculoid leprosy, fulminant aggressive hepatitis B virus (HBV), hepatitis C virus (HCV), or HIV infections leading to acquired immune deficiency syndrome (AIDS).

T and B cell responses are initiated according to the following general rules defined by antigen structure, antigen localization, its dose, and how long antigen is available (5, 7a). (The rules are summarized in Table 1.)

1. Conventional immune responses of T and B cells can be induced only in organized secondary lymphoid organs (i.e., lymph nodes, Peyer's patches, and the spleen).
2. T cells react against cell-associated antigens that are localized in secondary lymphoid organs in sufficient amounts and for a period of at least 3–5 days. Antigens that always stay outside of secondary lymphoid organs are

immunologically ignored. At the other end of the spectrum, antigens that are always in primary or secondary lymphoid organs—such as serum proteins—induce and delete all potentially reactive T cells. This process is called negative selection (24–27).

3. B cells react against highly repetitive, rigidly ordered antigenic determinants with shortlived IgM responses independently of T help, particularly if combined with a polyclonal activator (28). These antigens are called T-independent type I. Other multimeric but nonrigid antigens, including those on cell surfaces, will also induce B cell IgM responses (29) if presented together with indirect (or unlinked) T help (T-independent type II antigens).

Usually, B cells react against monomeric or oligomeric antigens only if structurally linked specific T help is provided (30). It is important to note that all B cell responses become dependent upon linked T help if antigen doses are limited (29). Also, the switch to long-lived IgG and the maintenance of IgG responses are usually dependent upon linked carrier-specific T help. The highly repetitive paracrystalline identical determinants on most infectious agents distinguish them from the usually mono-oligomeric self-antigens accessible to B cells (28).

A consequence of the tight T cell control of IgG responses is that B cells are in general not necessarily deleted by self-antigen. While they may not react against monomeric antigens, they are nevertheless potentially self-reactive. However, such autoreactive B cells are not readily induced to produce IgM or even switch to IgG responses because highly repetitive ordered self-antigens normally do not exist in the lymphatic system or in blood (31) and because self-antigens are usually not linked to polyclonal B cell activators (32).

Specificity is a key issue in any discussion about immune protection, immunological memory, and vaccines. The specificity of immunity is most directly measured by protection or cross-protection in vivo, e.g., protection by immunity against poliovirus strain I (serotype A) is absent against a subsequent infection with poliovirus II (serotype B) (Table 2) (33). Since both cytotoxic and helper T cells against serotype-defined virus groups are shared between the various serotypes, the obvious lack of cross-protection between serotypes (e.g., poliovirus I, II, III) in human populations indicates that only preexistent neutralizing antibodies and not primed helper or cytotoxic T cells are responsible for protection (Table 2) (7, 34–36).

Infectious agents that exhibit various serotypes are often highly cytopathic and cause acute diseases. Innumerable specificities of antibodies are usually induced by virus infection, but only neutralizing antibodies are protective (28, 37, 38); other antibodies, particularly those against internal viral antigens, are virtually irrelevant for protection. Those infections that tend to persist, including many viruses such as HBV, HCV, and HIV (23, 39, 40), facultative intracellular bacteria such as mycobacteria (9, 12, 41, 42), and intracellular parasites are usually controlled initially by T cells, but antibodies often play a controlling role also (e.g., 43, 44).

TABLE 2 Protection against serotype-defined infections

Virus	**Serotypes**	**Specificity of T cells: $CD4^+$, $CD8^+$**	**Neutralizing antibodies**
Polio	I, II, III	Largely shared Nonessential for protection	Highly specific Essential for protection
Experimental evidence			
Immunization	**Challenge**	**T cell response**	**Neutralizing antibody response**
Serotype A	Serotype B	Secondary anti-A and secondary anti-B	Primary anti-B

Measuring Immunity

How are immune responses best measured? This means measuring T cell and neutralizing antibody responses to assess essential mechanisms of protective immunity or so-called immunological memory and predict the efficiency of vaccines. Neutralizing antibody responses are measured in vitro by a virus plaque-reduction assay or by neutralization of the activity of bacterial toxins. These measurements correlate with the following observations: Neutralizing antibodies, usually of the IgG type, must possess an overall avidity of around $10^8\ M^{-1}$ or more and must be available in serum at concentrations of around 10^{-8} M ($\geq 1\ \mu$g/ml) to be protective in tightly controlled murine model infections (38, 45).

The protective capacity of cytotoxic T cells correlates best with the direct measurable lytic activity of lymphocytes in a 4- to 5-hour in vitro assay tested against infected target cells or target cells pulsed with relevant T cell peptides at concentrations of around 10^{-9} to 10^{-10} M.

The following tests done in vitro probably overestimate activities and relative precursor numbers of B and T cells: ELISA-binding antibodies assessing affinities or avidities of $<10^7\ M^{-1}$, T cells lysing targets pulsed with high peptide concentrations of 10^{-6} to 10^{-7} M, and T cells restimulated in vitro with high concentrations of peptides on antigen-presenting cells to reveal proliferation or intracellular interleukin staining. These assays reliably indicate priming and measure numbers of cells responding in buffered saline in vitro; but they yield only indirect correlates of their activation state and of protective immunological activity in vivo (Table 3).

Immunity can sometimes be monitored by injection of antigen intracutaneously in some infectious diseases. This skin test measures delayed type hypersensitivity (DTH) mediated by T cells. This DTH-reaction empirically reveals an immune status only for TB, leprosy, and perhaps sarcoidosis. Surprisingly, DTH-reactions against most viruses are not used (46). Why? A trivial explanation is that the

TABLE 3 T cell memory and persistence of infection: infection-immunity

	Localization or spread of infection or vaccine	Persistence for	Infection immunity
BCG	Subcutaneous lesion; regional lymph node	1–3 years	≤3 years
TB	Granuloma in lung and lymph node	Lifelong	Lifelong
Leprosy	Inapparent, tuberculoid, or lepromatous	Lifelong	Lifelong

DTH-antigen is not antigenic or is degraded too fast. An alternative explanation indicates that preactivated T cells are needed for DTH. Activation of T cells by the intracutaneously injected antigen is usually insufficient; no skin swelling reaction develops unless the infectious antigen has been persisting in the host, as is the case in chronic granulomatous infections that are associated with high levels of activated T lymphocytes. Conversely, readily inducible DTH probably signals persistence of antigen linked to an active infectious process and infection immunity (Table 3).

IMMUNOLOGICAL MEMORY

Immunological memory is defined by the finding that a primed host reacts more rapidly and with higher titers of antibodies or T cells to a second antigen exposure. This memory status correlates with increased precursor frequencies and enables the system to respond quickly and efficiently to a second exposure. The nature of the memory status correlates with the acquisition of numerous surface molecules on lymphocytes, but overall memory is still incompletely understood (3–6, 7a, 47, 48). Importantly, these parameters of immunological memory do not necessarily correlate with protection against reinfection. Therefore, an alternative possibility is that immunity is a low-level antigen-driven immune response that keeps T cells activated and maintains protective antibody titers. This would mean that protection by immunity eventually disappears when antigen disappears. These two views—inherent special quality versus antigen-driven response—differ fundamentally, and it is important to understand how protective memory functions.

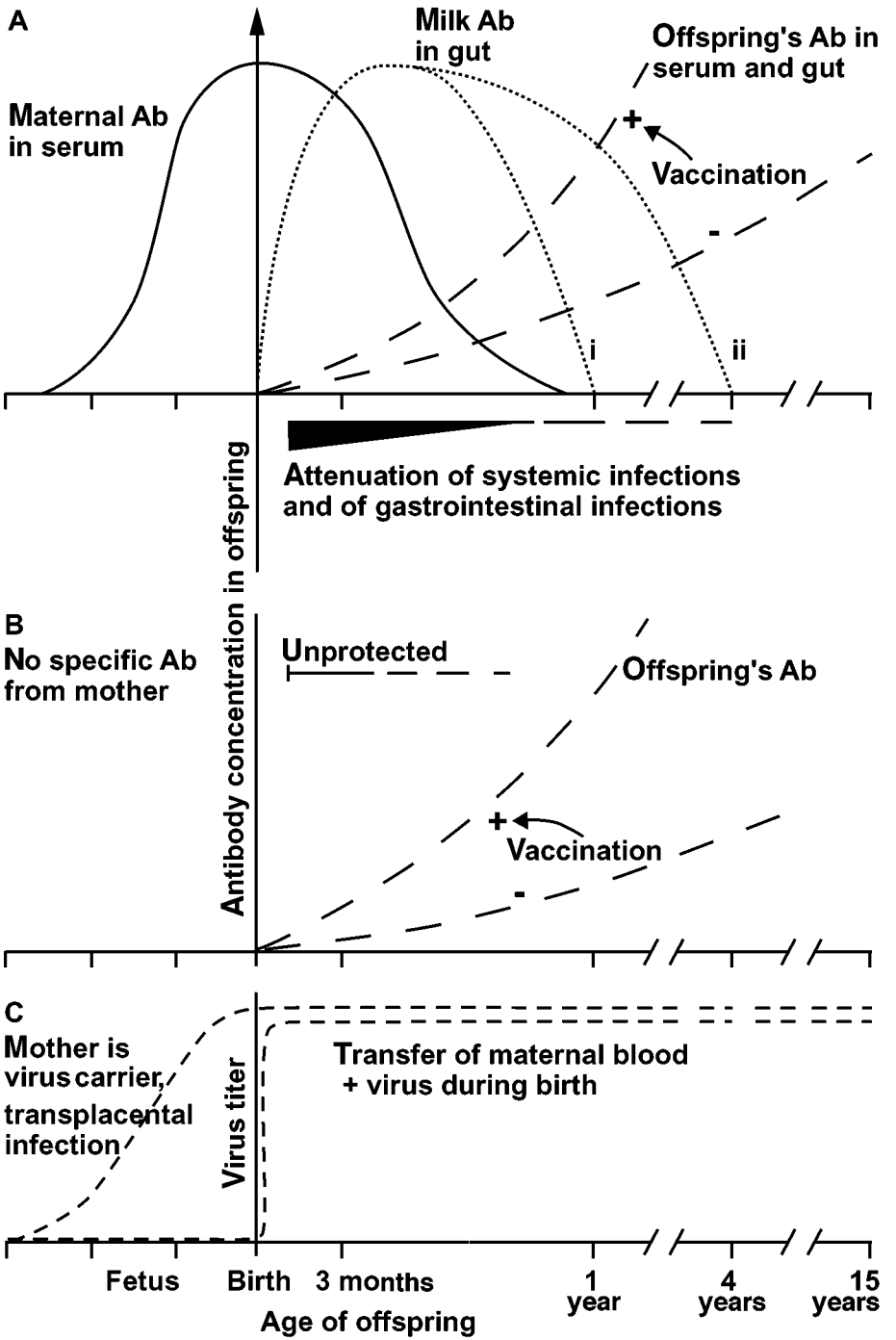
What Kind of Immunological Memory and Why?

Once humans have been infected with measles, pox, polio, or numerous other viruses, they are subsequently resistant against the same infection (34, 49). This immunity apparently correlates with so-called immunological memory. Many years of immunological inquiry and experimentation have been spent on this interesting phenomenon (2–5, 33, 48, 50); however, only rarely has it been analyzed from an evolutionary point of view. The period of life before and after birth may be

the evolutionary key to understanding immunity and immunological memory (5, 7, 7a, 51). During these periods of physiological immuno-incompetence, protective maternal immunological memory is essential for the survival of the fetus, the newborn, and the infant, that is, the survival of the species (see Figure 1). Here (probably as an oversimplification), we assume that if a naïve adult host does not survive a first infection, he does not need memory, and if he survives, he will not necessarily need immunological memory to survive a second infection. Of course several additional but less directly life-extending benefits of functional immunological memory can easily be stated, including improved fitness and herd immunity (see below) (10, 33, 52).

The passively acquired immunoprotection of neutralizing antibodies is absolutely required pre- and postnatally for two reasons (Figure 1): First, the embryo and infant are immuno-incompetent. Therefore, transferable immunity is probably an essential precondition in vertebrates (fishes, birds, and mammals) for maturation of the immune system. Second, transferable immunity (borne by longlived IgG) attenuates infections and permits active "vaccination" under optimal coverage and conditions.

Coevolution of infectious agents and MHC polymorphism has prevented easy selection of highly cytopathic mutants capable of evading MHC-restricted T cell recognition. On the other hand, MHC polymorphism has endangered immunological maternal-fetal relationships during ontogeny: The potential development of graft versus host or host versus graft reactions between mother and offspring is reduced by lack of MHC antigen expression in the placental contact areas, by general immunosuppression of the mother, and by virtually complete immunodeficiency of the offspring until birth (53–55). Protective antibodies in the serum of the mother are passively transmissible soluble forms of immunological experience. They protect the offspring for as long as it needs to develop its own T cell competence and to generate its own T helper cell–dependent protective and long-lived neutralizing IgG antibody responses. As discussed later, a fundamental role of maternal antibodies is to attenuate infections to permit a "physiological vaccination" of offspring. Coevolution of transmissible antibodies is probably an essential basis for the development of MHC polymorphism and MHC-restricted T cell–mediated immunity. This implies that the development of cytopathic agents that could not be controlled efficiently by adoptively transferred antibodies during this critical period of immuno-incompetence would not have been permitted because such infections would have endangered survival of the species (53, 56). Infants, incapable of generating their own immunoglobulins, are protected by maternal antibodies for the first 3–9 months after birth. Antibody is transferred via placenta (but not via milk) to the serum in humans. Human milk antibodies are active within the gut and influence the gut flora, at least before weaning. In contrast to humans and mice, calves are born without serum immunoglobulins, because maternal immunoglobulin cannot be transported through the completely double-layered placenta. During the first 18 h after birth they take up colostral maternal immunoglobulins via the gut. Gut epithelia transport immunoglobulins to the blood only in this short period. If


A
Milk Ab in gut
Offspring's Ab in serum and gut
Maternal Ab in serum
+
Vaccination
-
i
ii
Attenuation of systemic infections and of gastrointestinal infections
Antibody concentration in offspring
B
No specific Ab from mother
Unprotected
Offspring's Ab
+
Vaccination
-
C
Mother is virus carrier, transplacental infection
Virus titer
Transfer of maternal blood + virus during birth
Fetus
Birth
3 months
1 year
4 years
15 years
Age of offspring

this does not happen, calves will remain without maternal protective antibodies and die of various infections during the next few weeks. Their own yet immature immune system cannot act quickly enough to mount protective immune responses.

How can the antibody levels in human serum and milk be induced and kept high enough to provide protection for the offspring (7, 7a, 21, 51)? Protective antibody levels that cover all relevant infectious diseases cannot be generated during the 270 days of a human or the 20 days of a mouse pregnancy. Such infections would threaten the survival of the embryo, the newborn, and the species. In fact, cytopathic infections during pregnancy must be avoided as they can cause abortion or developmental abnormalities [these include rubella (57) and TORCH syndrome (58)]. The high level of immunity to such infections throughout a species usually conceals the enormous importance of this problem by making such infections rare. Herd immunity describes the equilibrium between susceptible and immune individuals in a population and a species. It depends on the infectious agent (acute or persistent), on the level of immunity (neutralizing antibody titers and/or activated T cells), on the population density and migration, and on animal reservoirs. Thus immunological memory at the individual level depends also on herd immunity at the population level.

All life-threatening acute infections must be survived by mothers before puberty. From this point of view, classical childhood diseases represent the coevolutionarily balanced infectious disease experience before procreation commences, and immunological memory represents accumulated immunological experience and protection before pregnancy (10, 33, 52). A host can die only once from infections in real life, usually through infections during the early period after birth. The important role of maternal antibody transfer has consequences for the health of the mothers. Since heavy (H) and light (L) chains of immunoglobulin are not encoded on the X chromosome, immunoglobulin levels and antibody-borne immunity cannot be an exclusively female characteristic. Nevertheless, immunological memory

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Figure 1 Role of maternal serum and milk antibodies (Ab) in protecting offspring. (*A*) Maternal antibodies transferred transplacentally protect offspring and attenuate systemic infections during the first 6–12 months after birth. Early (*i*) or late (*ii*) weaning influences attenuation of gastrointestinal infection. (*B*) If there are no specific antibodies in maternal serum this protection is absent. (*C*) If the mother is a virus carrier, neutralizing antibodies are not present and therefore the virus may be transferred transplacentally (in the case of lymphocytic choriomeningitis virus) or at birth by transfusion (HBV, HCV, HIV). In parts *A* and *B*, note that the offspring's antibodies start to be produced slowly within the first weeks after birth but provide effective protection only at 3–6 months of age; during this time active vaccination will enhance generation of specific antibodies by offspring. Protective maternal milk antibodies influence the gut flora and attenuate gastrointestinal infections; this depends on the period of nursing, usually relatively short (*i*) in Western countries but prolonged (*ii*) in developing countries.

transmissible from mother to offspring is regulated by important hormonal influences that improve overall antibody responsiveness in females compared to males. This responsiveness increases the transfer of maternal IgG to offspring, but it also correlates with the 5:1 ratio of autoantibody-dependent autoimmune diseases suffered by females compared to males.

On the Relationships Between T and B Cell Memory Versus Immunity

What is the role of T cell memory? Neutralizing antibody responses against related but serotypically distinct viruses are limited, not by primed T helper cells or cytotoxic T lymphocytes (CTLs), but by the precursor frequency of the specific B cells (Table 2) (35). Memory T cells cannot be transmitted from mother to offspring because of mutual immunological rejection. Therefore, why should long-term cell-mediated memory be needed? Two aspects must be discussed here: (*a*) the role of specific T cell–mediated protective immunity, and (*b*) the important role of immunity that depends on ongoing low-level infections, which includes the so-called specific infection-immunity and nonspecific concomitant immunity (5, 12, 59–61). Antibody-dependent memory cannot be sustained by shortlived IgM antibody because of its very short half-life of only 1 to 2 days (Table 1). In addition, because of the lack of receptors and its large molecular size, IgM cannot be transmitted to offspring via placenta or milk. The switch from IgM to IgG requires primed T helper cells and prolongs the half-life of antibody to about 3 weeks. Additionally, IgG is more diffusible and transportable via various Fc receptors and, crucially, this includes transport to the offspring. The next question to ask is how is immunological memory in the form of increased antibody levels or activated protective T cells maintained.

Is Immunity Dependent on or Independent of Antigen?

B cells cannot differentiate and mature to become antibody-producing plasma cells in the absence of antigen (62–64). B cells process antigen bound to surface immunoglobulin in order to present the relevant peptides on MHC class II molecules on their surface and to receive signals from specific T helper cells. This process is necessary for B cells to mature to plasma cells, but it is not sufficient to prime naïve T cells. Naïve T helper cells are efficiently induced only by antigen-presenting cells (APC), including dendritic cells (DC) presenting helper peptides via MHC class II. After priming, increased precursor frequencies of specific T and B cells are readily demonstrated in humans or mice (2, 5, 6, 65), but primed T and B cells without specific antigen are not by themselves protective, as shown in adoptive transfer experiments (Table 4) (29, 66). Protection requires preexistent neutralizing antibody titers, which are produced only by antigen-triggered B cells maturing to plasma cells. Some experiments have suggested that plasma cells may have a very long half-life, on the order of 150 to 300 days (64, 65, 67). However, this experimental evidence is flawed because antibody responses against nonprotective antigens composed of multiple undefined determinants have been

TABLE 4 Antigen dependence of protection by antibodies or by adoptive transfer of primed T and B cells

Adoptively transferred	Nonreplicative antigen added during transfer	Neutralizing antibody titer on day 3 after transfer	Protection against disease by cytopathic virus
Unprimed T + B	−	<1/40	−
	+	<1/100	−
Primed T + B	−	<1/40	−
	+	<1/100	−
Serum from immune donors	−	>1/1000	++
		Increased CTLp on day 0*	**Protection against immunopathological consequences of infection**
Primed CTLs	−	10–30×	−
	+	30–100×	++

*CTLp, cytotoxic T lymphocyte precursors

used for such studies (reviewed in 64). Protective antibody titers usually decrease over time [e.g., against diphtheria, tetanus toxins, or measles vaccines (68, 69)]. All these observations show that protective neutralizing or opsonizing antibody responses are antigen dependent (Table 4).

How is cell-mediated immune protection and protective T cell–mediated memory maintained and what is its role? Many experiments in mice have demonstrated that adoptively transferred $CD8^+$ T cells protect against acute infections by noncytopathic viruses or tumors. Under special conditions such protection experiments have also been successfully done with cytopathic viruses, such as with influenza virus in mice (70). But as stated earlier, neither humans nor mice are efficiently protected against distinct serotypes of viruses despite primed memory $CD8^+$ and $CD4^+$ T cell specificities. This strongly indicates that such T cell responses cannot efficiently protect across distinct serotypes. In fact, if T cells are acutely activated, they can exhibit a protective phenotype during the period of activation. For $CD8^+$ T cells specific for acute virus infections such as rhabdo- or influenza virus, this period lasts for only about 3 weeks (71–73). Here it is necessary to point out that primed memory T cells are reactivated to become protective effector T cells only by antigen in lymphatic organs (reviewed in 64). Therefore, any experimental protocol that brings great amounts of antigen into spleen and lymph nodes (such as by infection of mice intravenously or intraperitoneally with 10^6 plaque-forming units (pfu) of nonlytic virus, as opposed to a highly cytopathic virus that would kill the host) reactivates many effector T cells within 8–16 h. This reactivated T cell response is then rapidly protective. In contrast, the same virus initially infecting the same primed host strictly extralymphatically (e.g., via skin, mucosa, or olfactory nerve) will require either preexisting primed T cells to quickly eliminate newly infected peripheral cells or antibodies to prevent mucosal infection

or systemic spread. Strictly peripheral extralymphatic reinfections are the usual route of a natural challenge infection and reveal the relatively slow activation kinetics of primed T cells more accurately. Experience with many infectious diseases, including tuberculosis, leprosy, and perhaps HIV in a few seronegative AIDS-resistant patients, demonstrates that T cell memory provides efficient protection against reinfection from within and without. Importantly, however, this protection is clearly antigen driven, and is relevant for the individual host and the herd, but only indirectly important for the offspring. Infections controlled crucially by T cells are largely non- or low-cytopathic, or variably so (e.g., herpesvirus) (23, 74, 75), often slow in kinetics, and have a tendency to persist. These infections will not kill the host rapidly, but rather tend to establish a balanced state of infection-immunity. This term describes the coexistence of low numbers of infectious agents together with an active immune antibody and T cell response. Such a condition occurs in granulomata [for salmonellosis, TB, or leprosy (59, 60)] or for low-level infections of peripheral, nonlymphatic cells or organs [including the infection of neurons by herpesvirus, of kidney cells or lung epithelial cells by cytomegalovirus, and perhaps of β-islet cells or heart myocytes by coxsackievirus (76, 77)]. These few infectious foci are well balanced and controlled by an active, ongoing immune response in the host. This response against TB or leprosy is seemingly mediated exclusively by T cells, but against salmonella, coxsackievirus, herpesvirus, arenavirus, and probably rubella; the response also includes neutralizing antibody (Figure 2) (43, 60, 74, 78, 79).

Many non- or weakly cytopathic agents, such as HBV, HCV, or HIV, are transmitted before birth transplacentally, such as with lymphocytic choriomeningitis virus (LCMV) in mice, or more commonly during birth via maternal blood (Figure 1). Because the offspring are immuno-incompetent and because maternal immune defenses against these agents have obviously failed, viruses are best transmitted during this period of immuno-incompetence without endangering the survival of offspring and therefore also not of the host species. Some of the persistent noncytopathic infections may eventually cause serious disease late in the host's life, such as primary liver carcinomas 40 years after HBV infections; other agents may cause some chronic autoimmune diseases or chronic immunopathologies (e.g., AIDS). These consequences of chronic persistent infections usually show up much later than necessary for the species to procreate and survive.

Alternatively, infectious agents are transmitted orally early in life via peripheral mucosal or epithelial infections (herpes, CMV). For such variably cytopathic latent virus infections, transmission is early after birth under the umbrella of transferred maternal neutralizing antibodies that attenuate the infection and enhance establishment of a persistent infection that usually is clinically without serious consequences.

Maintenance of Immunity

High titers of neutralizing protective antibody, primarily in mothers but also in males for herd immunity, are essential to guarantee survival of offspring and of

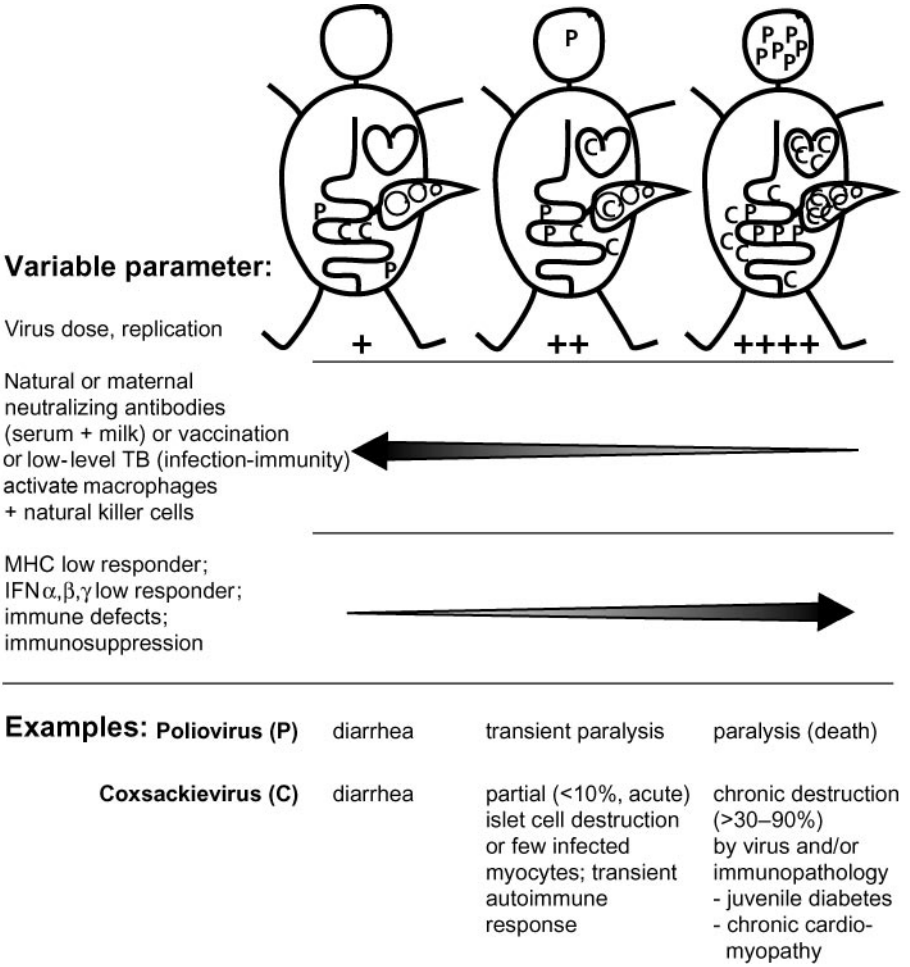

Figure 2 Influence of relative virus dose over time and of resistance mechanisms of the host on disease.

the species. Such maintenance of high titers of neutralizing antibody reflects three sources of reexposure to antigen. First, reexposure to the antigen can occur from external sources, a route typically used by poliovirus. A recent example is reinfection via a vaccine-derived mutant virus (80). However, the spread of the Sabin vaccine strains within households, schools, and via public swimming pools keeps immunity boosted. As discussed below, before vaccinations were frequently done, periodic subclinical reinfections starting early in life under the umbrella of maternal antibodies (33) boosted individual and herd immunity. Second, reexposure can occur from antigen sources within the host. This mechanism is essential for

understanding immunity against TB, leprosy, herpesviruses, HBV, HCV, HIV, and many parasites. An interesting case is measles virus that persists in the host not in a replication-competent form but as crippled virus apparently missing a functional matrix protein (81). The fact that children infected with wild-type measles on rare occasions develop subsclerosing panencephalitis (SSPE) shows that SSPE is just the worst case (that is virtually completely prevented by vaccination). Recently, testing by polymerase chain reaction using one single probe revealed a positive signal in 25% of autopsies of patients >60 years old who had been exposed to wild-type measles virus during childhood (82). Similarly, HBV virus in humans (83) or LCMV virus in mice (84) persists at very low levels and boosts immune responses of T and B cells repeatedly (44, 85). Third, antibody-antigen complexes on follicular dendritic cells are maintained for long periods (86, 87) and boost antigen-specific B cells directly as well as T helper cells indirectly. Since cross-priming and cross-processing of inert antigens can only exceptionally access the MHC class I pathway even in dendritic cells (88), these antigen depots are in general neither capable of maintaining activated CD8 T cells nor eliminated by CTLs. In the absence of antigen boosts, antibody responses eventually dwindle; this includes neutralizing antibody responses against tetanus toxin, diphtheria toxin, and inactivated polio vaccines. This again indicates that long-lived plasma cells alone cannot be responsible for maintenance of protective antibody titers. Taken together, T and B cell responses are regulated by structure, dose, localization, and duration of availability of antigen.

VACCINES AND IMMUNITY

Vaccines represent one of the greatest successes of medicine. Interestingly, all working vaccines protect hosts via neutralizing antibodies. This includes the classical childhood vaccines against bacterial toxins, measles, poliomyelitis, and smallpox. Vaccines in general do not prevent reinfection but reduce it so as to prevent disease (8, 9, 11). Thus, sterile protection probably is not—or is rarely—achieved with any of the vaccines. A clear example is vaccination against poxvirus. In the early 1900s, an epidemic in Boston revealed that about 20% of vaccinated children were not protected against reinfection (72). Von Pirquet evaluated how long a child vaccinated with vaccinia virus was protected against a retake of a challenge vaccination into the skin by scarification. The study revealed protection for only about 3 weeks (71, 72). Of course, he could not check neutralizing antibodies and viremia in these children, but he did not report on generalized disease; although indirect, this fits with the experience that revaccinees usually do not develop encephalitis. These clinical experiences therefore are compatible with the following interpretation: First, protective T cell–mediated memory is short-lived if the infection or vaccine is completely cleared. Second, neutralizing antibody memory that prevents hematogenic spread of the same virus is long-lived. The question of whether and what antigen maintains this response is discussed above.

TABLE 5 Vaccines and protective immune mechanisms

Available/successful		
Efficient	**Not completely satisfactory**	**Not available/not successful**
Diseases		
Smallpox	Measles	Tuberculosis
Poliovirus I, II, III	Mumps	Leprosy
Rubella	RSV	Parasitic diseases (malaria, leishmaniasis, schistosomiasis)
Tetanus		HCV
Bacterial toxins		HIV
Protective mechanisms		
Neutralizing antibodies	Neutralizing antibodies plus T cells	Activated T cells (plus neutralizing antibodies in some cases)

Successful Vaccines

All vaccines that work and provide proven protection for the individual as well as the population are vaccines that induce neutralizing antibody responses of apparently long duration after up to three booster injections (Table 5). Vaccines that do not work satisfactorily and do not induce long-term protection include vaccinations against TB, leprosy, and most classical parasitic infections, but also against some viral infections, including herpes, papilloma, and human immunodeficiency viruses. These infections have in common that neutralizing antibodies alone are not sufficient to eliminate or control the infection because infections persist extralymphatically in neurons, epithelial cells, or granulomas. However, as mentioned, these infections are by themselves either non- or weakly cytopathic (HIV, leprosy, etc.) or usually not efficiently lethal (e.g., herpesviruses) to the immunocompetent host. The efficient control of virtually all these agents requires T cell–mediated effector mechanisms in addition to protective antibodies. One interesting exception is HBV, against which neutralizing antibodies do protect very efficiently. This DNA virus is much less subject to variability when compared to highly mutable RNA viruses that represent pseudospecies. Therefore, a polyclonal neutralizing antibody response generally protects well against HBV.

Importantly, T cell–mediated protection against TB or leprosy usually depends on constantly activated effector T cells to control reemergence, spread, and expansion of the persistent infection (59). Whereas CD4 T cell memory may be maintained by inert nonreplicating antigen stored as immune complexes on follicular dendritic cells or in granulomas (if the agent persists or the antigen is poorly digestible and mixed with lipids), high levels of protective $CD8^+$ T cell memory depend on persistent infection and T cell activation. As stated above, the reason is simply that the MHC class I pathway of peptide loading generally depends on

intracellular synthesis and generation of peptides (29, 89). With special tricks this rule can be overcome, but in general this is very difficult to achieve and cannot reverse the general rule (88, 90–92).

Cytotoxic T cells have the key function of controlling noncytopathic virus-causing extralymphatic infections in peripheral solid organs. Although protective during the period of acute infection, these T cell responses may also be detrimental because of immunopathological destruction of otherwise innocuously infected host cells. Therefore, unbalanced cytotoxic T cell responses against too many nonlytically infected host cells cause disease, and in extreme forms death, and therefore have been selected against (5, 23). This delicate balance between immunoprotection and immunopathology is well illustrated in the various phenotypes of leprosy and in diseases caused by HBV infection. HBV-infected people may have inapparent infection (low virus, very efficient immune response) or apparent infection with either rapid recovery or fulminant hepatitis or chronic, aggressive hepatitis (high virus and either variable, quick, or slow T cell responses, respectively) caused by immunopathology in about 1–2% of HBV-infected patients. An extreme result is the establishment of a clinically inapparent virus carrier state (much virus, little or no T cell response) (5, 23).

As stated earlier, 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 mainly function to prevent viral spread within an individual host. An efficient early response limits both virus-induced and immunopathological disease. If virus is controlled down to low levels, chronic disease does not develop, or is retarded. If, however, virus has spread—or spreads again—widely, severe immunopathological disease may develop (Table 3). A similar balance exists in leprosy ranging from inapparent infection and controlling immunity to tuberculoid (immunopathological) and to the extreme polar form (immunologically virtually unresponsive) lepromatous leprosy. In all these examples few would question the evidence that low-level infection maintains protective immunity or that ongoing immune activity maintains infection at low levels. Mackaness coined the term infection-immunity, also called infectious immunity, to describe this important coevolutionary equilibrium (12, 41, 59, 60). Interestingly, chronic infection-immunity states, which include not only mycobacteria but also EBV, CMV, HSV, and many others, are accompanied by a heightened degree of macrophage activation via interleukins (IFNγ and TNF) and probably activated natural killer cells (12, 93). This status of concomitant immunity enhances basic nonspecific initial handling of many other low-level infections. Therefore, concomitant nonspecific infection-immunity not only has the benefit of controlling the specific infection but may also contribute considerably to improving overall natural or innate host resistance (16, 94). Therefore, such chronic low-level infections as exemplified by mycobacteria and many parasites represent an exquisite coevolutionary symbiotic balance of mutual benefit.

From all this we conclude that immunological memory represents not necessarily special characteristics of lymphocytes, but the results of coevolution of

infections and hosts. Protective memory seems to reflect low-level responses driven by antigen that is either stored as immune complexes on follicular dendritic cells; or reencountered from within, from persistent localized infections, such as granulomas or a few infected cells in peripheral solid organs (central nervous system, kidney, lung, parotid gland); or reencountered from outside infections. Although antibody memory is of key importance to transfer protection and attenuation to the immuno-incompetent offspring, activated T cells (often combined with antibodies) are important to control persistent infections within the individual host. Vaccines that imitate this coevolutionary situation of acute cytopathic agents and induce neutralizing antibodies have been very successful so far (Table 5). As mentioned, these include the vaccine for HBV, a noncytopathic DNA virus controlled very well by neutralizing antibodies. Those vaccines that aim at providing T cell–mediated memory and protection have in general not been satisfactory because they have not been able to imitate the key characteristics of infection-immunity; they are usually not persistent at clinically inapparent low levels of infection for long enough within the host to constantly activate protective effector T cells, particularly class I MHC-restricted $CD8^+$ T cells.

Vaccines That We Do Not Have

What we need, therefore, is long-term persistent vaccines that maintain immunity, without causing disease, against TB, leprosy, HIV, and HCV, variably combined with neutralizing antibodies (Table 5). Vaccines should guarantee periodical or continuous generation of MHC class I–presented peptides in secondary lymphoid tissues to activate $CD8^+$ T cells against peripherally located intracellular infections. They should also provide sufficient antigens to be taken up by APCs and macrophages to activate T cells to release IFNγ, TNF, and other interleukins that activate effector macrophages (12, 93). Attempts to achieve this with so-called attenuated vaccine strains have either offered only time-limited protection, such as bacille Calmette-Guérin (BCG) vaccine offers for a few years in small children (95), or have not been successful, such as vaccines for leprosy or against highly variable RNA-pseudospecies viruses like HCV and HIV that escape T and antibody responses constantly (see below). All indications are that the vaccine does not persist long enough for long-term immunity (or is too pathogenic), as illustrated in the following examples.

TUBERCULOSIS The efficiency of BCG as a vaccine against tuberculosis has been questioned, first in India and later in other populations (9, 12, 95). There is no doubt that BCG provides some protection for infants, but this protection is limited in duration and does not seem to extend to adulthood. Therefore, vaccination with BCG during the early period may have some overall beneficial effect against disease in children. Nevertheless, the concept of providing lifelong protection by vaccination is not borne out by BCG. This correlates with the fact that BCG does not persist in humans for much more than 3 years (Table 3). Therefore, BCG eventually

loses its capacity to keep specific effector T cells and nonspecific macrophages activated. This is in contrast to wild-type TB that persists in the host for the rest of the host's life (9, 12, 96). It has been shown in many instances that persistent low-level TB infection is responsible for exacerbation of infection during periods of immunosuppression, including old age.

In view of the nature of protective cell-mediated infection-immunity, a complete eradication of TB from a host is probably not really wanted (41, 59, 97). A constantly activated low-level specific and nonspecific immune response not only controls the internal spread of persistent infection but also renders the host resistant against reinfection from the outside. This view contrasts with the conventional opinion that immunological memory is antigen independent and that the complete eradication of TB from the host is the goal (9). As pointed out above, this view wrongly assumes that the specific memory T cells should be protective independent of low levels of infectious persistent antigen. Although the notion of infection-immunity may be less objectionable for mycobacteria, for many other persistent infections it is often hidden behind the term of latent or undetectable infection.

HIV Cell-mediated immunity including CD8 T cell immunity against HIV is rightly considered an important component of protective immunity (40, 98). While CD8 induction is efficient during phases of infection, this T cell response may eventually be reduced by exhaustion and/or by mutations within the CTL epitopes of the virus. Attempts at attenuating virus to elicit protective immunity against Simian immunodefieciency virus (SIV) in monkeys showed initial success. This attenuated persistently infecting SIV-vaccine strain protected for a prolonged period against infection with virulent SIV strains, but eventually through accumulation of mutations it also caused immunodeficiency disease (99), although somewhat later than is usually the case with the nonattenuated virus. Therefore, in one way this is a successful vaccine, although it is not as beneficial as we wish it to be. These results contrast with the excellent benefits that we are accustomed to with vaccines against measles or poxviruses, where protection is improved not just by a few years but is often lifelong. Nevertheless, the example of the attenuated SIV, and more recently of neutralizing antibody responses induced in monkeys before SIV infection (100), demonstrates on the one hand that unfavorable balances between persistent infections and the host can be shifted favorably. On the other hand, these examples point out clear difficulties for developing efficient vaccines against diseases usually controlled by infection-immunity.

The past six years have offered additional evidence in HIV patients that correlates with infectionimmunity, furthering our understanding of persistent infection and of its role in continuous T cell stimulation. By efficient antiviral chemotherapy, HIV loads have in some patients been suppressed to very low levels, which seemingly were not sufficient to maintain high levels of activated T cell responses. In these cases, the measurable cytotoxic T cell responses decreased and in some of these patients even disappeared (101, 102). This observation has led to the concept of repeated interruptions of chemotherapy to allow virus to reappear, so as

to repeatedly boost T cells and perhaps neutralizing antibody responses (that are not, however, routinely monitored!) (100, 103–105). The intention is to have the patient accumulate and boost an efficient immune response to eventually control infection for a prolonged time, without selecting for escape mutants and without continued antiviral chemotherapy. This is quite tricky.

Genetic or DNA vaccines (106, 107) including recombinant infectious agents that are well adapted, e.g., TB or HSV, or LCMV, may perhaps come close to providing protection against herpesvirus, TB, leprosy, HBV, HIV, and LCMV by eventually offering excellent imitations of the natural balanced situations of infection-immunity. Although we are not there yet, the prospects are not hopeless.

Postexposure Vaccines Against Infections and Tumors

As mentioned, antigen may stay outside of lymphatic organs and therefore be ignored by the immune system. Papillomaviruses (108) are excellent examples not only for a strictly peripheral viral infection but also for peripheral solid tumors. Attempts at using various vaccines to immunize against papillomaviruses and the tumors they induce exemplify the difficulties of the immune system in dealing with such strictly peripheral extralymphatic events. First, antigen does not reach draining lymph nodes, or reaches them late and via phagocytosis only on MHC class II. Second, even after T cells and antibody-producing cells are induced, antibodies have difficulties in reaching the peripheral areas. In addition, CTLs are often induced poorly or not at all, and activated T helper cells may not reach the tumor or the peripheral infection in sufficiently large numbers to eradicate the modified cells.

Because of the peripheral localization of papillomavirus infections and solid carcinomas or sarcomas, cytotoxic T cells are induced only rarely or late (109). The reasons are that viral tropism precludes macrophages and antigen-presenting cells from being actively infected, and therefore class I–presented epitopes of such viruses may not reach draining lymph nodes. Infected skin epithelial cells themselves usually cannot reach draining lymph nodes because they are localized outside of the draining pathways. Cross-processing of infected skin cells by Langerhans cells in a manner such that some of the infected skin cell antigens do reach class I has not been convincingly demonstrated. Although theoretically this could happen [particularly in vitro (88, 110)], the evidence that this is an efficient pathway in vivo is lacking (90, 109). Arguments made for warts here can also be made for other peripheral solid tumors, including many carcinomas, sarcomas, and melanomas. In all these processes, induction of effector CTLs at high enough levels to eradicate the tumor may be induced inefficiently and too late to be successful (109, 111). Although unconventional processing of phagocytized antigens onto class I MHC (called cross-processing and cross-priming) is a currently favored postulate, it is more likely that the following applies: Trauma and therapeutic interventions cause release of a few infected cells that can reach draining lymph nodes and induce $CD8^+$ T cells directly (109). The question then arises, is it possible to induce T cells if the system has not yet been primed, or is it possible to boost

the T cell immune response sufficiently to eradicate peripheral tumors? Clinical experience and model tumor situations suggest that priming of T cells alone is not sufficient to successfully eradicate even very small tumors. Only highly repetitive immunizations with a class I–presenting vaccine may induce, amplify, and sustain an effector T cell response that can control peripheral solid tumors (111). One major difficulty seems to be that even solid tumors of from 10^7 to 5×10^7 tumor cells (representing a volume of only 10–50 μl) require an enormous antigenic stimulation repetitively for the immune response to catch up with strictly peripheral tumor growth even under experimental conditions. Thus, the successful postexposure vaccination against solid tumors in patients requires enormous quantities of immunizing vaccines in repetitive short intervals very early to avoid great tumor cell numbers (1 ml corresponds to 10^9 cells) and escape mutants. Of course, cytostatic treatment, irradiation, or other cell-reducing therapies improve the balance in favor of protection. Unfortunately, however, the immune response is usually also impaired in parallel by these treatments. Thus, postexposure vaccination against peripheral tumors and peripheral persistent infections—at least at the level of effector T cells—seems very demanding and therefore difficult.

Vaccines with Occasional Problems

During the past 20 or 30 years several vaccines have been criticized because in relatively rare cases they provide only partial protection. They prevent systemic disease but a few vaccinees still develop partial disease as so-called breakthroughs (13, 112). Examples include particularly vaccines against paramyxovirus infections, i.e., mumps and measles (13, 14, 113). Measles virus vaccines are usually very efficient in the Western world but may be only partially efficient in very young children in developing countries. Attempts at increasing measles virus vaccine doses to accelerate and improve vaccine protection have resulted in a considerable increase in complications (112); some children even died of a delayed measles-related disease in the 12–36 months after vaccination. This result has immediately caused the reintroduction of low-dose vaccinations despite their overall lower efficiency. The details of the complications of the high-dose measles vaccine are not fully understood and the World Health Organization is studying the problem. It is nevertheless interesting that an infection of wild-type measles virus usually causes a lifelong neutralizing antibody response against measles despite the fact that no productive infection can be demonstrated in the host or in isolated populations of less than 100,000–500,000 (33). As mentioned, good evidence has accumulated over the past few years that a crippled measles virus may persist lifelong in the central nervous system (81, 82) and perhaps in other organs. This internal antigen source may well be responsible for boosting protective antibody responses. Whether measles vaccine strains can achieve a similar type of persistence is unclear (68).

A second problematic vaccine is against mumps. This vaccine has been introduced relatively recently, and the problem is again one of attenuation, of dosing,

and of protection against local versus systemic mumps virus infections. Interestingly, the so-called vaccine failures all represent infections only of the parotid gland, without accompanying orchitis or encephalitis or other systemic consequences of a mumps infection (13, 113). These results suggest that even a lower strength mumps vaccine generates a protective level of neutralizing antibody in the circulation, which then prevents systemic spread of the virus after relatively harmless peripheral reinfection via the parotid. An additional major reason for the breakthrough of disease in a few mumps vaccinees is probably that parotid infections are readily detected clinically. Mumps virus infection of the parotid is a very peripheral event, initially controlled efficiently probably via local IgA antibody that may not be induced optimally by our conventional, low-dose vaccines given subcutaneously. However, and this is very important for the future, the impact of such partial protection on herd immunity and on protection of offspring is yet unknown, and is discussed in the next section.

Role of Hygiene Standards and Transferred Maternal Antibodies in Childhood Infections

Finally, an important aspect is the crucial role that the very early host-parasite equilibrium, set up during the first few days of infection, plays for the rest of the individual's life. In the present context the key question is: How important is the level of transmissible protection via maternal antibodies for the susceptibility to infection 6–24 months after birth and for the present overall equilibrium between infectious disease and the human species? Maternal antibodies have often been discussed with respect to protection of offspring after birth (Figure 1) (5, 7a, 53, 56) and their impairment of vaccinations (33, 114–117). Here an attempt is made to put passive immunity by maternal antibodies in a much wider evolutionary context. Under conditions of reasonable levels of herd immunity, high levels of neutralizing antibody titers in mothers are transferred to offspring and do not necessarily protect completely against infection (i.e., do not sterilize), but do attenuate any infectious disease during the first months of life and thereby provide optimal conditions of active immunization. Under the high hygiene standards of the Western world, and the rapidly changing standards in developing countries, exposure to infections decreases and is delayed during life. Therefore, induction of protective antibodies and their maintenance are hampered. In addition, inadequate early (i.e., before pregnancy) and long-term upkeep of neutralizing antibodies may be due to less than maximally efficient vaccines and neglected revaccinations, in addition to reduced natural reexposure. Maintenance of high-level antibody titers will eventually influence herd immunity and the level of protective antibody titers in mothers. The latter will influence the overall susceptibility of offspring to childhood infections over time. Although active vaccination with live attenuated or inactivated vaccines will compensate for these developments, this will not apply to infections against which vaccines are not available or are not yet considered important (see below on infectious agents in immunopathologies and autoimmune diseases).

This concept explains not only why all species with a "modern" immune system (i.e., fishes, birds, and mammals) adoptively transfer immunity to their offspring but also explains the drastic impact that so-called emerging or new diseases have had previously and today on susceptible and unprepared host populations. Accordingly, the excessive morbidity and mortality of emerging infections may largely result from the lack of coevolution of passive protection during childhood infections during the period where maternal antibody could have attenuated early infection (33, 118) and therefore would have attenuated clinical disease. This aspect may be of extreme importance in a very general way in the future and needs careful monitoring at epidemiological and individual levels for the following reasons. Infection in early childhood, when preexisting levels of maternal antibodies attenuate disease, reduces susceptibility while installing protective immunity (Figure 1). If such an immunological handing-down of infectious disease experience from mothers to offspring influences overall disease susceptibility in the next generation, then vaccinations that are as efficient as wild-type infections, particularly long-term vaccinations (at least covering the reproductive period), may become of crucial importance; they will influence not only the survival of offspring but also generally of the species. The relevance may readily be seen from experience with poliovirus infections (Table 6). Because poliovirus-neutralizing antibody levels are determined by previous infections, by vaccine exposure, and by natural or vaccine boosters, increased hygiene standards have caused and contributed to the later and later occurrence of polio infections, first in the Western countries but now also in the rest of the world (33). The consequences are that maternal antibodies do not protect adequately for sufficient periods against these infections. Late infections are therefore not attenuated and cause more severe acute disease symptoms. Similar problems may be projected eventually for measles, mumps, and many other infections against which antibody levels in mothers are still sufficient to protect them but insufficient to passively attenuate infection in offspring. It must be kept in mind that childhood vaccinations have not yet revealed their effectiveness and influence across more than one or two generations (79, 118–122). From this point of view vaccination and global vaccine strategies are no longer a problem of the developing world alone, but may, in a true evolutionary context, become of utmost importance also in medically and hygienically "overdeveloped" Western countries. Unless our vaccines are improved and vaccination schedules and disciplines are stringently adhered to, progress in controlling infectious disease may turn into a nightmare.

TABLE 6 Poliomyelitis: age distribution in Massachusetts 1912–1952

Years	0–4 years (%)	5–9 years (%)	10+ years (%)
1912–1916	70	18	12
1930–1934	28	38	34
1948–1952	18	27	55

Modified from Reference 131.

Hygiene Standards, Passive Maternal Protection, and Autoimmune Disease

If the above considerations are biologically important, then humans have entered a rather dramatic new environment. Hygienic standards prevent early and sufficient exposure to many infectious agents. The same infection is different later, at times when maternal passive protection has waned (Table 6; Figure 2).

In addition, relatively lower antibody titers in maternal transferable protection may not protect offspring for sufficiently long periods. Overall, this has at least two potential consequences. First, adoptively transferred passive protection from mothers to offspring during the early months after birth may be inadequate if the baby is not itself vaccinated efficiently and if the disease remains at high enough prevalence. Importantly, this passive protection also influences encounters with many other infections not usually considered to be life-threatening during the first few years of life. We have argued that unbalanced excessive T cell immunity against noncytopathic infections causes immunopathological disease. If the inducing infection is unknown (123, 124) or is unrecognized because it is experienced by most people (123), the unbalanced immune response may well be classified as autoimmune disease. The potential influences that hygiene status, exposure to infections at certain ages, and passive as well as active immunity may have on susceptibility to immunopathological and autoimmune disease are illustrated in the following examples.

JUVENILE DIABETES The following scenario may explain what these influences could mean in terms of overall balance between infectious agents and immune protection versus slow or chronic degenerative or immunopathological disease (Figure 2). Let us assume that diabetes type I is—at least in some patients—caused by virus infections such as coxsackie B virus (the same argument applies to similar types of infection). Let us postulate that maternal antibodies, because of limited exposure, may not be sufficiently high in serum and/or milk for long enough periods to protect the offspring after birth against widely spreading coxsackie B virus (125). In addition, partial or no maternal nursing transfers incomplete or no protection against gastrointestinal infections. Since coxsackie B virus infections are not usually lethal, variations in overall spread from the gastrointestinal tract to other endodermally derived structures, including islet cells in the pancreas, may vary greatly (Figure 2). The preexisting level of neutralizing antibodies from the mother in serum and via milk in the gut, including spontaneous or natural antibodies (Figure 1) (7a, 116, 118), influences the extent of gut infection and overall hematogenic spread and distribution of viruses. This protection may either be prolonged or nonexistent depending on nursing practices. In addition, the young host's immune response is influenced by the MHC. Therefore, passive protection, MHC and hygiene standards, and the time of the first few effective infections have a direct influence on whether coxsackie B virus (77, 126) may eventually reach islet cells (Figure 2) or heart myocytes (see next paragraph). The few cases of

acute insulitis resulting in acute diabetes may just represent one extreme phenotype. This spectrum is comparable to acute severe measles encephalitis versus subsclerosing panencephalitis at the two extremes, or alternatively to inapparent infection versus paralytic or lethal disease for poliovirus. The implication is that virus may reach islet cells and either cause their excessive destruction directly or via immune destruction of islet cells. Self-antigens are thereby released that have been immunologically ignored up to that point because these self-antigens usually do not reach draining lymph nodes at sufficient levels. Prolonged release of virus and of so far ignored self-antigens to secondary lymphoid tissues may induce autoimmune T cell and possible autoantibody responses, particularly if this virus infection persists for longer than usual. The potential consequence of chronic immune responses against self-antigens inducing new lymphoid follicular structures in the target organ is discussed below. Taken together, the history and quality of passive protection, and the timing of exposure to infection (due to hygiene conditions or active vaccination) may drastically influence not only infection kinetics but also the extent of immunopathology and autoimmunity. Can the general increase of juvenile diabetes, and of the delayed type in India, and perhaps even part of the increase of Type 2 diabetes in the elderly, be explained by important shifts in these key parameters together with a probable further exacerbation by nutritional factors?

DILATED CARDIOMYOPATHY AND COXSACKIE VIRUS INFECTION A similar argument may explain virus-dependent dilating cardiomyopathies (Figure 2) (127–129). Depending on the acquired levels of protective antibodies in serum, the overall relative distribution of a virus may vary from a few to considerable numbers of myocytes becoming and remaining infected. Immunopathological T- and antibody-dependent destruction and chronic inflammation then may cause an inapparent or a more severe cardiomyopathy resulting in heart dilation, insufficient function, and death. Again, as for juvenile diabetes, host MHC and overall antibody levels at various times of life, including positive maternal antibodies in serum and gut in infants, may greatly influence such otherwise unnoticed disease patterns. Therefore, increases in disease incidence may well be linked to the complex relationships between herd immunity, maternal antibody titers, and hygiene status.

Similar arguments could be made for several immunopathologies and autoimmune diseases where known or unknown (123) infectious agents may trigger initial autoantibody or autoaggressive T cell responses against so far immunologically ignored self-antigens. If such immune responses are maintained, they often result in neoformation of lymphatic organs within the target organs (130). This process in turn maintains the autoimmune disease chronically, because the so far ignored self-antigens now persist locally in an immune response inductive environment. Thus, these cases represent a reversal of the conventional process. Antigen is eventually no longer brought into lymphoid organs, but lymphoid organs are "brought" into the antigen-expressing peripheral tissue. Since self-antigen usually

cannot be eliminated, the immune response is maintained as chronic autoimmune disease until all cells are destroyed (burned out). Examples include rheumatoid arthritis, Hashimoto's thyroiditis, juvenile diabetes, and Sjögren's disease.

Taken together, these examples and explanations—although yet unproven as a pathogenic principle directly for many cases—suggest by correlation that overall evolutionary balances that had been equilibrated under wild-type evolutionary conditions for thousands of years have perhaps altered rapidly and dramatically in the past 50 years. Whereas 200 years ago neither hygiene standards nor preventive and analytical medicine nor antibiotics were the standard, this has changed drastically. Perhaps this rapid change will reveal some coevolved equilibria that have now changed too dramatically within one or two generations (a very short time in evolution). If so, the overall greatly prolonged life and reduced childhood disease for many of our generation may result in disadvantages for the coming generations. From this point of view, it is important to understand the true nature of immunological memory: Is immunity (i.e., immune protection) by immunological effector T cells and neutralizing antibodies antigen independent, or is it dependent on persistence by antigen, as the evidence reviewed here strongly suggests?

CONCLUSIONS

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 coevolution 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 vertebrates is immature at birth, particularly of fishes, reptiles, and birds. Successful vaccines have been those that can imitate the generation of neutralizing or opsonizing antibody responses that seem to be the only limiting factor against acutely cytopathic agents. In contrast, cell-mediated immunity against infections that persist in the host is much more difficult to imitate. This is largely because the balance between attenuation on one side, and persistence of the infection to provide constant stimulation of protective effector T cell responses on the other, so far has not been achieved with vaccines to a level of perfection similar to the coevolved balance between host and infectious agents. Similar problems are posed by classical parasites, which in their coevolution over time have come to innumerable sophisticated balances with their hosts that will be considerably more difficult to imitate, or beat, even compared to TB, leprosy, or HIV. But the aim should be—and must be—to develop strategies that aim at exactly that perfection of low-level persisting infectious agents exemplified by TB, HIV, HCV, HBV, many herpesviruses, and most classical parasites. Although this will not be easy (witness the limited effects of BCG vaccines), the development of persistent

genetic vaccines, including persistent recombinant infectious agents, may bring us closer to such a goal.

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