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REVIEW

The use of antibodies in the prophylaxis and treatment of infections

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The use of antibodies to provide passive immunity to infections has a long history. Although the coming of antibiotics greatly reduced its use for bacterial infections, it is still widely used for a variety of purposes which are reviewed here. The use of animal antisera gave way to the use of human convalescent serum as a source of antibodies and more recently human and monoclonal antibodies have become widely used, not just providing passive immunity but as therapeutic agents. The current uses of antibody therapy are discussed as are the problems of antibody-mediated immunopathology and how this can be avoided. More recent developments include the making of monoclonal antibodies that react with cross-reacting determinants on flu viruses. Such antibodies are not usually made following infection and they provide a very promising approach to providing passive immunity that will be effective against a variety of different strains of the flu virus. It is also pointed out that passive immunotherapy can act as a surrogate vaccine providing that the subject gets infected while protected by the passive antibodies. Finally, there is a section on the possible use of oral antibodies given as food to prevent diseases such as infantile gastroenteritis.
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INTRODUCTION

The adaptive immune response has both a cellular and humoral element and the relative importance of these caused much controversy already in the nineteenth century.[1] It is now recognized that T cells are the important mediators of the cellular adaptive immune response, whereas antibodies are those of the humoral response. It is also clearly true – though more argued about than perhaps it should be—that both arms of the immune response normally act together, and that dissecting too minutely which element is involved in a particular phenomenon is of more interest to the immunologist than to the body *in vivo*.

Experiments in animals or 'experiments of nature' in man ablating one element of the immune response have been widely used to discover the types of infectious disease the subjects become prone to. Such studies appear to give quite clear answers. Antibody deficiencies, for example Bruton's agammaglobulinaemia, give rise to pyococcal infections and not, in general, to unusual virus infections. There are exceptions where the virus has to pass through the circulation from its primary site of infection to its secondary target site, as is the case with polio and hepatitis viruses. On the other hand, T-cell deficiencies, for example, the di George syndrome, are associated with unusual sensitivity to viral infections and not to bacterial infections. Nevertheless, sterilizing immunity to viruses is brought about by antibodies and not by T cells.

This emphasizes the distinction between the body's response to infection and the response aimed at by vaccination, both active and passive. The immune response that has evolved to fight infections is (at any rate after maternal antibody has decayed towards the end of the first year of life) geared to clearing infections after they have occurred. The ability to recover from a virus infection is undoubtedly largely due to T-cell destruction of virus infected cells before they can produce more replicating virus. On the other hand, immunity to bacterial infections is largely concerned with the generation of suitable levels of inflammation, with enhancement of phagocytosis and of the intracellular killing of the bacteria. With bacteria which live predominantly extracellularly, like the pyococci, these effects are largely mediated by the humoral immune system. For bacteria that live within cells (*Mycobacterium tuberculosis* being a classic example), the cellular immune response is required to produce granulomas and to produce the cytokines that activate macrophages to kill the bacteria.

The aim of prophylactic immunisation is, wherever possible, to prevent the establishment of infection. For virus infections, the object is to achieve sterilizing immunity by preventing viral entry into host cells. Because most virions (except those of retro and lentiviruses) express no major histocompatibility complex they are not seen by T cells and only antibodies can produce neutralisation. Vaccination has proved highly successful in achieving sterilizing immunity for many important viruses and this protection is mediated by antibodies alone. With bacterial infections, the situation is more complex. Although patients with agammaglobulinaemia suffer severely from pyococcal infections, making effective vaccines against these organisms has frequently been difficult. There is still no good vaccine against *Staphylococcus aureus* or against *Streptococcus pyogenes* and the much improved conjugate vaccines against pneumococci have only recently

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been introduced. For eukaryotic parasites, whether unicellular or multicellular, the situation is more complicated still and there are as yet no licensed vaccines.

Research into immunisation against bacteria was slowed down considerably after the introduction of antibiotics which were originally believed to provide a complete solution to the problems of bacterial infection. The rapid and progressive growth of antibiotic resistance has shown that this belief was false and that the need for immunological approaches to deal with bacterial infections will become increasingly important. While current vaccines aim to prevent cell entry or to enhance phagocytosis or intracellular killing, newer strategies are now being explored. These include the use of antibodies modified for purposes such as delivering drugs to microorganisms in highly concentrated form or recruiting local T cells by using bispecific antibodies.

There is one situation where immunity to disease is clearly mediated by antibodies alone and that is those due to the secretion of exotoxins, of which diphtheria and tetanus are the classic examples. The use of antibodies to combat these diseases is very long standing and is indeed where the use of passive antibody really began. Emil von Behring was the given the first Nobel Prize in Medicine for the development of anti-diphtheria toxin antiserum, which was in its time a great medical advance.

The citation for this prize read: ''for his work on serum therapy, especially its application against diphtheria, by which he has opened a new road in the domain of medical science and thereby placed in the hands of the physician a victorious weapon against illness and deaths''. This victorious weapon is still being used more than a century later and the ways in which it can be applied have been greatly extended.

THE HISTORY OF PASSIVE IMMUNISATION

After the introduction of anti-diphtheria toxin, other anti-toxin antibodies followed soon after. Prominent among these was anti-tetanus which has continued to be used ever since, and antibodies against the toxins of haemolytic Streptococci, Shiga dysentery and gas gangrene. These antisera were originally made in horses and it was horse serum and, later, fractions containing immunoglobulins that were used. Antibacterial antisera were also made. Prominent among these were antibodies to *Streptococcus pneumoniae* (pneumococcus) which until the advent of sulphonamides and antibiotics was the only available treatment for pneumococcal pneumonia. Antisera against *Neisseria meningitidis* and against Leptospira were also used though probably with less effect.

Passive immunity was also used against virus diseases. Horse antisera were raised against polio, influenza and canine distemper.

Convalescent human plasma was used to treat some patients in the great influenza pandemic of 1918—apparently with some good effect.[2] More recently,[3] reported using convalescent plasma to treat patients with H1N1 flu.

From the 1930s, convalescent human sera were used for the treatment of measles and yellow fever. To be effective against virus infections, it is generally necessary to give the antibodies during the incubation period and anti-measles antibodies were used to protect exposed individuals who were not immune, particularly pregnant women, immunodeficient children and children with leukaemia on corticosteroids.

The use of horse antiserum did give rise to considerable problems because horse serum is highly antigenic to humans. There was therefore the possibility of anaphylactic reactions (where immunoglobulin E antibodies had been formed) and of serum sickness (where immunoglobulin G (IgG) antibodies had been formed). Because of the substantial volumes of serum injected, there could be enough antigen persisting after antibody formation to allow a reaction even to a first injection. Serum sickness is a systemic reaction involving IgG immune complexes, whereas the local effects of IgG immune complexes are known as Arthus reactions. To most modern immunologists, Arthus reactions are small red lumps that occur at vaccination sites and they are regarded as trivial. However, Arthus reactions following the intramuscular injection of several ml of horse serum, as were given to treat Pneumococcal pneumonia, could be extremely destructive and cause considerable muscle necrosis at the injection site. For these reasons, the use of horse—and of other animal—antisera, fell into disuse. Probably the only animal antisera that are still used are those against snake venoms where human antibodies are not yet generally available. However, animal antisera were used from their first use in diphtheria in 1891 until the 1930s when the use of human plasma and then immunoglobulins from either convalescent or immunized subjects came into general use. Human immunoglobulins carry dangers of their own. They do not normally cause severe Arthus reactions, but they can transmit infections and it was many years before effective procedures for rendering antibody preparations virus-free were developed. The major dangerous virus contaminants were hepatitis viruses—both Hep B and Hep C—and, since the 1980s, HIV.

Human immunoglobulins are still used widely for several purposes. The first is for the treatment of children with antibody deficiency states. These are treated with pooled human immunoglobulins from donors who have, between them, suffered from most of the infections to which these children will become prone. This treatment has been highly successful and is still used in situations where more direct interventions, such as bone marrow transplantation, have not been carried out. Second, human immunoglobulin for short-term use was also used to protect travellers going to countries where certain illnesses, particularly hepatitis A, are endemic. Now that a vaccine against hepatitis A is available this is done much less frequently. Third, and slightly peripheral to the topic of this review, is the use of intravenous immunoglobulin preparations to treat certain immunological disease, notably thrombocytopenic purpura and the Guillain–Barré syndrome. The mechanism by which intravenous immunoglobulin works in these circumstances is probably complex and still slightly controversial. Probably a mixture of non-complement fixing alloantibodies in these mixtures from many donors is responsible for protecting formed elements of the blood from attack from complement fixing autoantibodies. The immunoglobulins may also sequester certain activated complement components and block the activity of F*c* receptors.

MONOCLONAL ANTIBODIES

The field of antibody therapy was revolutionized by the discovery of monoclonal antibodies by Köhler and Milstein.[4] This technology allowed substantial amounts of individual antibody molecules to be made and advances in protein engineering allowed them to be modified, both to change their affinity to give them an entirely human framework even if they were originally raised from animals, and also to make such antibodies from phage libraries so that they need not necessarily correspond to any antibody that would be made spontaneously against an infective organism. The development of monoclonal antibodies into licensed prophylactic and therapeutic agents took many years, but monoclonal antibodies are now an extremely important form of therapy, not only in the prevention and treatment of

infections, but also in the treatment of tumours and the treatment of various inflammatory states such as rheumatoid arthritis. No definite limit has yet been found for the circumstances where they may be used.

It is really from the development of monoclonal antibodies that the revival of interest in passive immunisation has taken place. At the moment, most monoclonal antibodies are still made in diploid human cells. However, there are many techniques now described where they can be made in bacteria, in transgenic plants, in silk worm larvae, in the milk of transgenic animals[5] and the egg whites of transgenic chickens.[6] None of these fascinating and innovative techniques have yet led to products that are used in man. This probably reflects largely the oppressive nature of the regulatory environment which makes the development of an antibody for therapy so expensive, but it is to be anticipated that such antibodies will come into use.

Another interesting recent observation is that camelid antibodies from camels or llamas contain antibodies whose specificity is determined wholly by the heavy chain which makes them much easier to engineer.[7] These camelid antibodies are also unusually heat stable.[8] While camelid antibodies are unsuitable for systemic use in man because of their antigenicity, their possible use for oral passive immunisation will be discussed in a later section.

ANTIBODY-MEDIATED IMMUNOPATHOLOGY

While antibodies are a prime way of neutralizing viruses and enhancing the phagocytosis of bacteria, they can also cause immunopathology. One example is the occurrence of immune complex disease where antibodies and bacterial or viral antigens to which they react are formed in sufficient quantity that they can produce serum sickness type reactions, or particularly immune complex nephritis. Post-streptococcal glomerulonephritis is a classic example of this where following, usually repeated, infections with Group A Streptococci the immune response is sufficient and immune complex nephritis results. A second mechanism of antibody-mediated immunopathology is the formation of autoreactive antibodies which cause tissue damage. A classic example is rheumatic fever which is again due to repeated infections with Group A Streptococci—in this case, only infections in the throat give rise to this complication for reasons which are unknown—and antibodies are formed which cross-react with heart antigens[9] and give rise to the syndrome of rheumatic fever. The extent to which this happens in other situations is controversial, but speculations have been made about the role of antibodies made to unknown infectious organisms cross-reacting with myelin and being involved in, for example, the Guillain–Barré syndrome which occurs occasionally after influenza, and very rarely after vaccination.[10,11] The possibility that a similar mechanism, but involving a variety of different candidate viruses, might be involved in multiple sclerosis has been suggested but never confirmed.

The third mechanism is where microorganisms have subverted the immune response and have devised strategies of entering cells through F*c* receptors when combined with antibody, i.e. they have converted a neutralizing antibody into a potentiating antibody. The best established example of this is in dengue where antibodies, particularly to different serotypes of the dengue virus, potentiate entry of dengue virus into macrophages. This causes a great increase in viral load and when high virus amounts are released into extracellular space containing high antibody levels, their reaction then gives rise to dengue haemorrhagic fever, probably by an immune complex mechanism.[12] It is possible using passively given antibody to avoid these complications by engineering antibodies to have F*c* regions that do not allow such effector mechanisms to occur.[13] For example, if one produces an antibody that does not react with macrophage F*cγ* receptors, then it will not potentiate infection in dengue, and the idea of engineering such antibodies is an attractive one for treating the early stages of this disease.[14] It is also possible to produce antibodies that do not activate the complement system, at any rate by the classical pathway, and will therefore have a greatly reduced ability to give rise to immune complex disease.

Passive antibodies can be screened and selected not to be autoreactive and it should be possible to check any antibody given therapeutically or prophylactically against an infectious agent to make sure that it does not react with peripheral myelin or with heart muscle or any other autoantigen. There is considerable potential in the use of engineered antibodies of this description, but considerations of the great expense of making such reagents have so far prevented any coming into routine human use.

ANTIBODIES NOT NORMALLY MADE IN INFECTIONS

There is little doubt that in the co-evolution of viruses and the immune system of the hosts they infect, mutual adaptations occurred. Viruses have developed strategies to deviate the immune response of their host to immunodominant epitopes, the response to which does not produce clinical immunity. This is known, for example, with HIV.[15] Analysing antibody responses to a series of peptides from gp120 has shown that those peptides to which the titres were highest in HIV patients also had lower peaks of activity in normals. When the sequence of these peptides was examined in the database, it was found that they were generally present in other viruses so that these responses seemed to have been secondary responses which are known to deviate antibody responses away from antigens seen the first time; and in this way, HIV succeeds in deviating antibody responses to antigens which are not directly involved in the invasion of cells or cell fusion.

This problem that infection may not give rise to neutralizing antibodies can potentially be overcome by the use of 'cascade immunisation'. This is a technique where antibodies made against a first course of immunisation, (i.e. those to the immunodominant epitopes), are then given passively to inhibit the antibody response to these epitopes when a second course of immunisation is given. This allows antibody formation to less dominant epitopes which may give better immunity. In theory, this procedure can be repeated for a third immunisation. Cascade immunisation was demonstrated by Taussig and Lachmann[16] using IgG as the antigen and showing that giving antibody to the immunodominant F*c* before immunisation with intact IgG allows antibody formation to F*d*.

While cascade immunisation is likely to be too complex for clinical use, it has recently become clear that there are potentially valuable antibodies that are not made after infection. The prime examples are the antibodies to the membrane fusion site of the flu haemaglutinin.

Passive immunotherapy to flu has been transformed by the development of monoclonal antibodies from phage display which react with the fusion site on influenza viruses and prevent infection not by conventional neutralisation but by inhibiting the fusion of the virus with the cell membrane. This fusion site is shared by a wide variety of influenza viruses, including H5N1 and H1N1. Two groups have independently described these antibodies.[17,18] These monoclonal antibodies both prevent infection *in vitro* and prevent death *in vivo* in an extremely impressive fashion.[19] Given during flu epidemics, they could provide a broadly reactive surrogate vaccine (see below) without the need to isolate a new flu variant and manufacture a new vaccine.

OTHER ANTIBODIES FOR USE IN INFECTIONS

Antibodies to cytokines

It has been long suspected that, for example, in influenza in 1918, much of the mortality may have been due less to the virus itself and more to the host response and there is a suspicion that some of this was due to cytokine storms.[2] It is therefore possible that antibodies to some pro-inflammatory cytokines might be useful in treating such cytokine storms. In a recent non-infectious example of cytokine storms—the trial of TGN1412 anti-CD28 antibodies—it was shown that tumor-necrosis factor rose very rapidly after these injections[20] and the possibility that anti-tumor-necrosis factor might be useful where such reactions occur is worth exploring. However, this is an approach which requires caution as of course these pro-inflammatory cytokines also play some role in resistance to infection.

Bispecific antibodies to recruit T cells

In situations where antibodies are ineffective on their own in containing the infection, it is possible to use them to recruit T cells by having a bispecific antibody, one combining site reacting with the infectious agent and the other to a T-cell antigen such as CD3. This approach has its origin in tumour immunology and there are successful reports of this approach in containing residual non-Hodgkin B-cell lymphoma cells in patients treated with bispecific antibody reacting with CD19 at one end and CD3 on the other. This is claimed to be a promising and successful therapy.[21] A similar approach has been suggested for localizing radionucleides to tumours[22] and could be adapted to localizing drugs at sites of infection.

Advantages of passive over active immunisation

Antibodies can be given after exposure to the organism, while vaccines are then ineffective.

Antibodies can be given to immunodeficient or immunosuppressed subjects who cannot be vaccinated. In HIV-infected patients, antibody therapy can provide immunity to other pathogens without giving rise to T-cell stimulation which promotes HIV growth.

Antibodies can be given where no vaccines are available, for example, Ebola or Marburg viruses, but convalescent plasma may be.

Antibodies as surrogate vaccines

There is a further possible advantage of giving antibody therapy if the patients subsequently become infected while still protected by the antibodies. This derives from the work of Renegar *et al.*[23] who found that IgG antibodies against flu protect the lung from disease but do not protect the nose from infection in mice, which only polymeric IgA was able to prevent. This would suggest that infection after giving passive IgG antibody would provide a form of surrogate vaccination, whereby the subject becomes actively immune without developing the disease. It is highly plausible that the vaccines currently used against seasonal flu that generate largely IgG antibodies act in a similar way. This would suggest that the effect of flu vaccination will be very different in those who become infected compared with those who do not.

ORAL PASSIVE IMMUNITY

Many infant animals acquire all their antibodies from the mother through the colostrum. However, after a few days, antibodies are no longer absorbed from the gut and oral antibodies then serve only to protect the gut itself. To exploit this latter purpose, transgenic rabbits, goats and cows have been made that secrete antibodies in the milk, but this has turned out to be very expensive and such products have so far only been used as biopharmaceuticals. However, an important advance has been the development of transgenic hens with oviduct specific expression of therapeutic proteins.[6] This is an extremely attractive technique since the transgenes are inherited stably from chicken to chicken and the breeding of transgenic chickens is very much easier than that of transgenic mammals. It would be, in addition, possible to use the remarkable properties of camelid antibodies for oral administration from transgenic ovalbumin. Camelid antibodies have their specificity directed entirely by their heavy chain[7] making their engineering much easier, but also have the remarkable property of being very heat stable,[8] so that camelid antibody transgenic eggs could be lightly boiled to sterilize them without destroying the antibodies. This technique would allow the production of antibody containing food—egg whites containing antibodies to verotoxin or to shigatoxin or to rotavirus—which could be given to children in sub-Saharan Africa where the deaths due to such infections are common.

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