

CURRENT STATUS OF ACTIVE IMMUNIZATION AGAINST HEPATITIS B INFECTIONS.

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For those of us working in the field of hepatitis B, the past 10-15 years have been extremely exciting ones, not only has the virus causing the disease been identified, but its precise physical chemical structure has been determined and its genetic material extracted and cloned. Sensitive scientific tests have been developed for detecting a range of antigens associated with the surface and core of the virus and both total and class specific antibody directed against them.

These tests enable us to detect virtually all acute or chronic infections, to study the natural history of the disease and to determine its epidemiology and mode of spread. The Hepatitis B Virus (HBV) has been transmitted to chimpanzees and these animals provide important models for studies of the pathogenesis of disease and for evaluating methods of prevention and treatment.

Finally, safe, effective vaccines have been developed which will prevent infection and reduce the incidence of the chronic carrier state. As a consequence, WHO has begun to plan a global control programme which has the potential of saving tens of millions of lives. It is hard to imagine a more exciting or rewarding period!

In discussing the current status of hepatitis B vaccines, one must first consider the discoveries which made the development of vaccines possible.

In my opinion there were three landmarks in this process:

1. Blumberg's discovery of the **AuAg** in 1963;
2. Dane's detection of the HB virion in 1970 and in 1971;
3. Krugman's demonstration of the protective efficacy of boiled serum.

In 1963 Barry Blumberg, an American geneticist, accidentally discovered a novel precipitating antigen in the blood of an Australian aboriginal. Blumberg had no idea of the significance of this finding and gave the antigen the deliberately non-committal name "**AuAg**". Five years later Fred Prince in New York and Dr. Okochi in Tokyo independently demonstrated that this antigen was a **marker** of the presence of the HBV, and was actually situated on the surface of the virus.

Manfred **Bayer** in Blumberg's laboratory examined the serum of a chronic carrier under the electron microscope (EM) and found it

contained large numbers of virus-like particles, measuring 22 nm in diameter and scattered tubular forms. However it was not until 1970, that the virus itself was visualized by David Dane, an Australian scientist working in London. As Dr. Blumberg has told you, the serum of patients who are acutely or chronically infected with HBV, contain three morphologically distinct particles:

- the large 42 nm spheres or Dane particles which have an outer coat and inner core containing nucleic acid (NA). These particles are the mature viruses and are fully infectious;
- the remaining 22 nm spheres and tubules which are usually present in great excess, contain neither cores nor nucleic acid and are non-infectious. They simply represent excess viral coat material produced during a relatively inefficient replicative cycle and are released into the blood.

The importance of this observation was not lost on either Blumberg or Prince, both of whom realized that it should be possible to separate the excess coat material and use it as the basis of a vaccine.

In 1971 Professor Krugman and his colleagues in New York demonstrated that serum collected from chronic carriers of HBV could be rendered non-infectious by boiling for one minute and that children who received three doses of this material were largely protected against challenge with live virus.

This was the starting point for the production of HB vaccine. Several research groups and commercial manufacturers began to devise methods for separating the 22 nm particles from the blood of chronic carriers. The most active groups were National Institute of Health (NIH), Merck Sharp and Dohme (MSD) and Pasteur, and of these, the latter two now have marketable products which are licensed in many countries.

Vaccine manufacture is now becoming big business because of the size of the potential market and vaccines are under production in Holland, Japan, Korea and China, while Singapore has just signed an agreement with MSD to produce their own vaccine locally.

The basis of all these vaccines is essentially similar, namely to take whole serum and separate from it the non-infectious 22 nm spheres and treat them to destroy any residual or contaminating virus.

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In discussing the current status of the vaccine, I will restrict my remarks to the two vaccines which are currently licensed:

1. the Merck vaccine, known in Australia as H-B-VAX, and
2. the Pasteur vaccine, known in Australia as HEVAC-B.

These vaccines are produced at special new facilities in **Pennsylvania** and Normandy, respectively. Unlike other viral vaccines which are traditionally produced from tissue culture, hepatitis B vaccine is produced from the blood of chronic carriers of the disease. This is a unique process for a human vaccine and requires a new set of requirements for vaccine manufacture, which are more like the requirements for production of a blood product than of a conventional vaccine.

To assist National Control Authorities, in 1981 WHO issued a set of requirements for HB vaccine. Earlier this year the WHO Expert Committee on Biological Standardization revised these requirements and a draft document has been circulated to manufacturers and testing authorities for comment. This document contains a number of controversial areas which are to be resolved at a meeting in Geneva in December, after which the revised guidelines will be issued.

In discussing the Merck and Pasteur vaccines, it is useful to look at how they fit in with the proposed guidelines. The WHO guidelines cover seven specific areas:

- manufacturing processes;
- selection of donors;
- tests on plasma and plasma pools;
- concentration and purification;
- inactivation;
- tests on purified inactivated batches, final bulk, final product;
- other - records, labelling, shipping, etc.

1. Manufacturing

The WHO guidelines for manufacturing procedures suggest that separate areas be used for manufacture and inactivation of the vaccines, that the staff work with only a single virus each day and that none of them should be chronic carriers of **HBV**, that there be restricted access to the laboratories and that the general guidelines of manufacturing and control authorities be adhered to.

Both manufacturers comply with the WHO guidelines, relating to the environment in which the vaccine is to be made. The only difference between the vaccines relates to the

consistency of production protocols. Whereas the Merck production procedures have remained unchanged for several years, the procedures used by the Pasteur Institute have undergone several modifications over the past 6 years. The WHO draft guidelines place a heavy emphasis on consistency of production techniques and regard any change, no matter how small, as effectively a new procedure whose safety must be formally established. Whether these strict requirements will be relaxed in December, is a matter for conjecture.

2. Selection of Donors

WHO recommends that only adults be used and their unforced consent obtained. All donors should be examined by a registered physician and found to have no history of acute or chronic diseases, a normal physical examination, to not be pregnant or have undergone surgery within the past 72 hours, or have been recently immunized with a live vaccine. They also suggest that the subject's haemoglobin (or haematocrit) and, if relevant, serum protein level be measured and demonstrated to be adequate prior to collection of their blood.

Both manufacturers adhere to these guidelines. Merck obtain the majority of their blood from New York blood donors with high titres of HBsAg, who are repeatedly plasmaphoresed. The Pasteur Institute uses single donors, most of whom are European. Donors are selected on the basis of the presence of HBsAg and the absence of **HBeAg**.

3. Tests on Donor Plasma and Plasma Pools

WHO recommends that individual units should be tested for sterility and the presence, titre and **subtype** of HBsAg. Pools of plasma also should be tested for the absence of extraneous viruses. Both manufacturers adhere to these guidelines using standard techniques including the inoculation of adult and newborn mice, embryonated eggs and a variety of cell cultures. The Pasteur Institute also test their plasma pools for reverse transcriptase and examine **them** by EM.

Merck produce their pools from approximately 40-60 repeatedly plasmaphoresed donors, while Pasteur's pools are obtained from single units of plasma obtained from approximately 400 donors.

4. Concentration and Purification

The WHO draft requirements suggest that the procedures which are used should have been demonstrated to concentrate HBsAg and remove the bulk of extraneous material,

that the protein content of the purified material be measured and compared with an International Reference Preparation, and that HBsAg should comprise at least 95% of the total protein present.

The major differences in the procedures used by the two manufacturers are as follows:

- Merck first concentrate HBsAg by precipitation with ammonium sulphate then concentrate the 22 nm particles by isopycnic banding in **sodium** bromide, followed by rate-zonal centrifugation through a sucrose gradient. The partially purified antigen is then enzymatically digested with pepsin at low pH and treated with **8M** urea to remove extraneous blood and liver components and subjected to gel filtration.
- The Pasteur Institute initially precipitate immune complexes and lipoproteins with 5.5% PEG and then precipitate HBsAg by the addition of 10% PEG. Washed pelleted HBsAg is subjected to rate-zonal centrifugation twice on self-formed sucrose gradients, and once on caesium chloride. **Antigen**-containing fractions from each gradient are analysed, concentrated by **ultracentrifugation** and pooled. The final step involves isopycnic centrifugation on a caesium chloride gradient.

At this stage both vaccines comprise an essentially homogeneous population of 22 nm particles.

The purity of each preparation has been demonstrated in a number of ways including U-V spectrophotometry, SDS-PAGE and analysis of amino acid composition. No major contaminants have been detected and both manufacturers believe that more than 95% of the protein in the final product is HBsAg.

HBV DNA and HBV specific DNA polymerase have not been detected in either vaccine.

5. Inactivation

Perhaps the biggest difference between the two vaccines lies in the procedures used to **inactivate** residual **live** viruses. Since this is a most controversial area, and has been used by some people as the major basis for selection of the vaccine, it is worth discussing in some detail.

The proposed WHO requirements state that "the inactivation procedures used should be demonstrated to be capable of inactivating all viruses", which clearly doesn't refer to tobacco mosaic virus or tomato bushy stunt!! In practice it is usually interpreted to mean a demonstrated capacity to inactivate representative members of all groups of viruses which

may be found in the blood and are capable of infecting man.

Merck use a three stage process consisting of pepsin at low pH, **8M** urea and formalin.

The Merck inactivation procedure has been demonstrated to inactivate representatives of all known groups of animal viruses including the slow viruses. In addition each of the three steps has been demonstrated to be individually capable of inactivating 10^5 chimpanzee infectious doses of **HBV/ml**. Treatment with pepsin at low pH will **completely** inactivate members of the rhabdo, pox, toga, herpes, corona and reovirus groups. Treatment with **8M** urea has been shown to inactivate these viruses and in addition members of the **myxo**, picorna and slow virus groups. **Formalin** inactivates a wide variety of viruses including members of the **parvo** and astrovirus groups and at least one of the agents of non-A non-B hepatitis.

The Pasteur Institute use a two stage inactivation procedure - centrifugation through caesium chloride and incubation with **1:4000 formalin** at **30°C** for 48 hours. Centrifugation through caesium chloride is claimed to reduce the titre of HBV by up to 10^4 and to inactivate enveloped viruses. There are good theoretical grounds for believing that the procedures used by the Pasteur Institute will either exclude or inactivate potential contaminating viruses.

The manufacturers estimate the efficacy of each step in removing HBV to be as follows: PEG 3 logs, sucrose gradients 5 logs, caesium chloride 9 logs, i.e. 17 logs. Even at a conservative estimate of 12 logs, this is vastly in excess of the known titre of the starting material.

In addition, the combination of techniques used should inactivate HAV, the delta agent, NANB, CMV, EBV, HTLV, retroviruses, **togaviruses**, bunyaviruses and perhaps slow viruses as well. Much of this data is recently derived: in view of concern in some quarters, it is important that it be published soon.

6. Tests on the Purified Inactivated Batches - Final Bulk and Final Product

The draft WHO requirements suggest a variety of tests for sterility and innocuity for the content of protein and HBsAg, for the presence of preservatives and adjuvants, the absence of pyrogenicity, for potency and identity and demonstration of safety in chimpanzees.

Both manufacturers comply with the draft guidelines but there are some differences in details, such as the final concentration of HBsAg in the vaccine (Pasteur 5 **ugm/ml**, Merck

20 $\mu\text{g}/\text{ml}$) and the number of chimpanzees used in the safety tests.

7. Other

The final requirements laid down by WHO relate to the keeping of records, labelling, distribution, etc., and are not really relevant to our current discussion.

In general, both manufacturers have taken advantage of the stability of HBsAg and its unique biophysical properties for the production of their vaccines. Both have placed considerable emphasis on physical separation of the 22 nm HBsAg particles by **ultracentrifugation** and added a variety of additional steps to inactivate residual or contaminating live virus.

The ultimate test of the safety and efficacy of a human vaccine comes from its widescale use in man. Both vaccines have now been administered to a variety of different **populations** using a variety of different doses and immunization schedules. As the American and French trials vary in many respects, it is impossible to perform a direct comparison of the two vaccines; however sufficient data is available to make a number of generalizations.

I think that from the information we have, it is now possible to state confidently that both vaccines are antigenic, effective in preventing infection with hepatitis B and development of the chronic carrier state and that both appear to be safe.

The Merck vaccine has been tested in a large number of children and adults and in groups of immunosuppressed patients. Most experience has been obtained with a three dose schedule in **which** 20 μg of HBsAg is given by injection at times 0, 1 and 6 months. In healthy adult populations about **40%** of subjects develop antibody after the first dose, 90% after the second. The **third** dose raises the seroconversion rate to 96-98% and results in an increase in the Geometric mean titre (GMT) of antibody.

There is now some unpublished data from the U.S. that healthy adults respond well to three 10 μg doses of the vaccine if it is administered intradermally, although the incidence of local reactions was higher than with larger doses given intramuscularly. If this data is confirmed, this may provide a much more economical way of administering the vaccine.

While immunization of adults is important, in developing countries control of hepatitis B will require a vaccine which is safe and effective in children, especially neonates. Several studies have been carried out both in the U.S. and overseas and several others are nearing completion.

As we heard from Dr. Beasley and Dr. Huang this afternoon, the vaccine is well tolerated in children and the newborn and three 10 μg doses are highly antigenic.

Merck currently recommends a three dose schedule (10 μg at 0, 1 and 6 months) for children aged from 6 months to 10 years. As far as I am aware the vaccine is not yet licensed for use in children aged less than 6 months, although clearly from data which is now available this restriction will soon be lifted.

Studies have also been performed in groups with impaired immune function such as **sero-negative** patients or chronic haemodialysis, using three 20 μg or 40 μg doses of the vaccine. The findings which have been published to date indicate that although most dialysis patients can be immunized, they do not respond as well as healthy adults – only 60-70% developing detectable levels of anti-HBs after three 40 μg doses. The immune response is worst among subjects over the age of 40 years.

Therapeutic efficacy has been demonstrated both **in** chimpanzees and in man. The most important study being that of Szmuness and his colleagues. In this randomized double-blind, placebo controlled trial conducted among promiscuous male homosexuals in New York, the vaccine was shown to be well tolerated, safe and effective. A number of important **observations** were confirmed in three other large studies.

Among subjects who received a full course of vaccine and developed anti-HBs, none developed clinical hepatitis B or acquired circulating HBsAg. The appearance of detectable anti-HBs after immunization indicates protection from disease.

Although HBV infection did occur among vaccinees, most occurred within 4-5 months of the initial injection and were probably contracted just before or just after the course began. Despite this, HBV vaccine reduced the incidence of acute hepatitis B by 92%. The vaccine appears to be safe – there being no evidence that it contains residual HBV, non-A non-B or other viruses. The incidence of side effects was acceptably low.

A small proportion of individuals (4% in the study) did not respond to the vaccine and remained as vulnerable to infection as recipients of the placebo.

The question has been raised as to whether these non-responders are people who would have become chronic carriers if they had been naturally infected with the **virus**. Long term follow-up of this group shows that they are

completely susceptible to HBV and have a normal chance of becoming chronic carriers (i.e. about 10%).

The duration of protection is unknown. Preliminary data shows that about 85% of people retain detectable levels of anti-HBs for five years and that the original antibody level is a good predictor of survival of antibodies. Although some people whose anti-HBs titres have fallen to undetectable levels have been reinfected with HBV, none of these infections have been accompanied by **antigenaemia** or disease. Obviously the need for and frequency of booster doses is an area which requires further study.

From a global point of view, control of hepatitis B depends largely upon the ability to prevent early childhood infection. As we have heard, there is now good data that the Merck vaccine is antigenic in infants and young children and that its administration early in life can reduce the chance of babies born to **HBeAg-positive** mothers becoming chronic carriers by 95%. There is no general agreement on the most effective and simplest protocol for preventing vertical transmission. We have heard the results of Beasley's study and there is also data from the United States, Japan, Hong Kong and Africa. When these trials and a large study in Burma are completed, the optimal regime will become clear, as will the need or otherwise for combining immunization with administration of hepatitis B immune globulin (HBIG).

THE PASTEUR VACCINE

After limited antigenicity studies in chimpanzees, the Pasteur vaccine was evaluated in a randomized placebo-controlled trial among staff members of French haemodialysis units.

Healthy seronegative subjects were given **3** injections of 5 μ g of vaccine or placebo at monthly intervals. The vaccine was found to be well tolerated, antigenic and effective. After one dose of the vaccine 18% of subjects developed anti-HBs (> 10 **Miu/ml**) and this rose to 60% and 94% after the second and third injections. Peak levels of antibody were detected 4 months after beginning the course.

The incidence of hepatitis B was greatly reduced in subjects who received the vaccine. As with the Merck vaccine, some infections occurred in vaccinated subjects but all of these were within 9 weeks of the first injection, presumably in people who were already incubating the disease.

A similar randomized placebo-controlled trial of hepatitis B vaccine was carried out in dialysis unit patients known to be at high

risk of infection. Although the same dose was used, neither the immune response nor the protective efficacy were as satisfactory as in staff members, only 60% of patients developing antibody compared with 94% in healthy staff.

In general, females responded better to the vaccine than males, as did subjects under the age of 50 years. Immunization produced a significant reduction in attack rate with 82% of the immunized group remaining infection free at 12 months compared with only 58% of those who received the placebo. The majority of infections in the immunized occurred prior to receiving the third dose of vaccine.

The Pasteur vaccine has also been evaluated for safety, antigenicity and susceptibility in infants and young children in West Africa. The vaccine appears to be antigenic, well tolerated and capable of preventing a large proportion of infections acquired early in life even when used in only three doses, one at birth, the second a month later, the third at 12 months. Maternal antibody does not interfere with the vaccine response. At present several studies are being carried out to determine the optimal dose and timing of immunization for prevention of vertical transmission of infection. Particularly important are the studies which seek to incorporate hepatitis B immunization into the general pattern of the WHO "expanded programme" of immunization.

In summary then, a review of the published data and information presented at this meeting supports the view that both the Merck and Pasteur vaccines are antigenic and effective in preventing hepatitis B infection in man. Both can be given without **ill-effect** to carriers of the virus or subjects possessing circulating antibody to the core or surface antigen and the Pasteur vaccine has been given to pregnant women without ill-effect. Comparable **sero-conversion** rates are achieved by three doses of each vaccine administered by a variety of routes and protocols. Antibody levels seem to persist for several years and are correlated with immunity to reinfection.

A handful of people fail to seroconvert to either vaccine. These people remain susceptible to infection and if infected, do not have an abnormal risk of becoming chronic carriers.

Both vaccines are safe in newborn babies and capable of interrupting vertical transmission of infection, usually in combination with HBIG. The most effective way of achieving this result is still under debate.

There is good data also that monovalent and bivalent vaccines are equally effective in

protecting against infection with the major subtypes of HBsAg.

SAFETY

The recognition of AIDS, a severe disease syndrome involving opportunistic infections and Kaposi's sarcoma, which is occurring with disturbing frequency in a number of countries, has caused considerable concern about the safety of hepatitis B vaccines. Although the cause of the disease has not been identified, there is evidence that it may be associated with a blood-borne infectious agent. As some of the plasma from which the hepatitis B vaccines have been produced has been collected from populations known to be at increased risk of acquiring AIDS, it is not surprising that concern has arisen about the safety of the vaccine. The data relating to the possibility of transmission of AIDS has been considered by a number of national authorities and by the World Health Organization, all of whom agree that there is no evidence to suggest that immunization with either of the vaccines carries an increased risk of developing AIDS.

Not only is the Merck vaccine produced by a method which is designed to inactivate members of all known groups of animal viruses, but surveillance of large numbers of vaccine recipients has failed to demonstrate evidence of transmission by the vaccine. In a two year follow-up no cases of AIDS have been recorded in more than 20,000 subjects who are at low risk of acquiring the disease and who received

batches of vaccine prepared from plasma collected since 1980. In addition, the incidence of AIDS among promiscuous male homosexuals who have received the vaccine is no different from the incidence amongst similar subjects who have not received the vaccine.

The Pasteur vaccine has been produced by a method which results in an extremely pure product and inactivated by procedures which will destroy most human viruses. Formal proof that the method currently used will destroy members of all known groups of animal viruses is lacking but more data will be available soon.

According to the manufacturers, more than 1 million doses of the vaccine have been distributed and more than 11,000 subjects who have received it are being carefully followed up. To date, no case of AIDS has been reported in a recipient of the French vaccine.

While I understand the attitude of some health care workers in developing countries, who are at low risk of infection and have a low risk of developing serious sequelae and who have elected to wait for the second and third generation vaccines, this is not an attitude which is acceptable in developing countries. On the basis of the Beasley data, we can calculate that every minute, a child is born who will die of the long-term sequelae of hepatitis B. To delay the introduction of mass immunization in those countries which need it on the basis of a hypothetical risk, is in my opinion unthinkable.