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REPORT OF THE WHO WORKING GROUP MEETING ON TOXOPLASMOSIS
 VACCINE DEVELOPMENT AND TECHNOLOGY

Fontevraud, France, 3 July 1992

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INTRODUCTION

Dr T. Fujikura, Veterinary Public Health, Division of Communicable Diseases, welcomed the participants (Annex I), and opened the meeting on behalf of the Director-General of WHO. He explained the purpose and scope of the meeting as follows:

- to review the public health significance of toxoplasmosis;
- to discuss the results of research on toxoplasmosis vaccine development, with particular reference to immunology, antigenic components for vaccine development, animal models and biological requirements of a vaccine;
- to discuss planning and management of field vaccination trials; and
- to elaborate work plans and international cooperation for 1992-1994.

Professor A. Johnson was elected Chairman, Dr Y. Suzuki Vice-Chairman and Dr Astrid Tenter served as Rapporteur.

1. PUBLIC HEALTH SIGNIFICANCE OF TOXOPLASMOSES VACCINE DEVELOPMENT

The current status of research in toxoplasmosis vaccines is as follows:

- it is possible to vaccinate animals against toxoplasmosis;
- live vaccines reduce the rate of disease (TS4, S48 and T263 in sheep, rats and mice; RH in pigs). However, their capacity for reducing/preventing cyst formation upon challenge should be further defined.

Live attenuated T. gondii vaccines are not likely to be acceptable for use in humans. Non-viable vaccines containing T. gondii epitopes could be used (1) in humans, (2) in meat animals to reduce disease and cysts in the environment, and (3) in cats to reduce oocysts in the environment. However, the use of each one may depend on the prevalence of infection in given geographic areas. Before meat animals and cats could be vaccinated there is a need for more information on the epidemiology of the parasite. The aim of vaccines is to stop disease and its transmission but they can probably only reduce it.

2. PARASITE ANTIGENIC COMPLEX FOR VACCINE DEVELOPMENT

Target epitopes have been studied with regard to their localization and function in the parasite and their demonstrated efficacy in animal models. A vaccine against toxoplasmosis is expected to limit the dissemination of the tachyzoite proliferative stage. During acute toxoplasmosis, tachyzoites are found either as extra-cellular invading forms or as intra-cellular replicative forms, so that the rationale for such a vaccine has to focus on the killing of both. The different "ecological niches" imply that different antigens have to be targeted to induce appropriate effector mechanisms.

The antigens identified for study include those located on the surface of toxoplasma, in dense granules, rhoptries and micronemes. Currently, putative candidates include SAG1, GRA1 to GRA5, ROP1 to ROP5, MIC1 and MIC2.

Those that have been most intensively studied and for which there is some evidence for protection are SAG1, GRA2 AND ROP2. However, other chemical moieties, e.g. carbohydrates and lipids, need to be investigated.

The relevant antigens may be presented in a variety of manners, e.g. as sub-unit vaccines, live recombinant vaccines, or peptide vaccines. The following avenues should be explored:

- expression of T. gondii proteins in intra-cellular organisms, e.g. Mycobacteria;
- selection and synthesis of peptide epitopes;
- potential for synergy among different antigens.

Preliminary expression experiments with SAG1 in Salmonella and GRA1 in Vaccinia have been inconclusive. A variety of adjuvants have been tested. Liposomes and non-ionic surfactant vesicles appear promising and stimulate cellular immune responses without the detrimental effects of Freund's adjuvant. A potential T. gondii vaccine should undergo three phases of development:

- safety and efficacy for laboratory animals and human volunteers;
- safety and effectiveness against congenital infection in a monkey model;
- effectiveness in a human immunity trial. Due consideration should be given to the possible low frequency of congenital toxoplasmosis in the selected population.

More information is needed on the epidemiology of toxoplasmosis, especially information on the prevalence of infection in relevant populations, analysis of the major routes of transmission and of strain heterogeneity, and mathematical predictions of the impact of vaccination.

3. CELL-MEDIATED IMMUNE RESPONSE TO A VACCINE

Cell-mediated immunity (CMI) may be more important than the humoral or secretory antibody. A combination of both may be involved in protective immunity. Cytolytic T cells are important in animal models and their role should be investigated further. Aims to be achieved are invasion blocking and killing of the parasite.

Many observations in animal models as well as in humans indicate that CMI is absolutely necessary for protection although it probably acts in concert with other immune mechanisms. It is thus important to define more precisely the different components and mechanisms of the CMI response against T. gondii. This will allow the definition of the best targets and antigen presentation systems to be used for vaccine development.

The consensus is that CMI responses are important in functional immunity, either directly through T cell(CD8+)-mediation or through production of appropriate stimulatory cytokines. In developing a vaccine it is important to evaluate stimulation of these cells (a) via in vitro assays,

to assess responses to parasite antigen and investigate MHC (major histocompatibility complex) restriction, and (b) in vivo animal models to evaluate functional protection.

CMI responses are accountable for both protective and inflammatory responses which can be a cause of diseases induced by T. gondii infection. It should be known which cells and cytokines are responsible for protection and disease. The aims of a vaccine are to induce protective cytokines and to prevent harmful cytokines.

Thus it is worthwhile characterizing the protective immune responses in humans and in animal models. Multiple effector mechanisms may contribute to protective responses. Some of these include secretory IgA and serum IgG antibodies, T lymphocytes which are cytolytic for infected cells, T lymphocytes and natural killer cells which produce gamma-interferon, and T lymphocytes which produce interleukin 2. Other possible relevant effector cells include macrophages, platelets, eosinophils or lymphokine-activated killer cells. The genetic restriction of this response can be used to define protective epitopes. It will be important to determine that harmful immune responses are not produced. The same epitope may elicit both a protective or harmful response depending on the mode of delivery. It will therefore be important to define epitopes, delivery systems and the immune responses they elicit in animal models and for humans, as well as to determine the ability of recombinant proteins to elicit potentially protective immune responses for multiple MHC haplotypes as there is considerable MHC polymorphism in humans. It will also be necessary to determine whether multiple proteins, and which ones, will be needed in vaccines because of this MHC polymorphism.

4. ANIMAL MODELS, CHALLENGE STRAINS, LEVELS AND TYPES OF IMMUNE PROTECTION REQUIRED

4.1 Animal models

The key to development of vaccines is definition of what should be accomplished in a specific host, e.g. to prevent oocyst shedding in cats; to prevent cyst formation in meat animals; to prevent initial acquisition by humans after birth or in utero by immunization of the mother prior to pregnancy. Different immune responses will probably be relevant to protection against oocyst formation versus congenital infection.

4.1.1 General considerations and current status. It is critical to distinguish between animal models that might resemble human infection (e.g. rat, pig, monkey, SCID-hu mice (severe combined immuno-deficient outbred mice) and animals - not really models - that in themselves serve as species to be vaccinated (e.g. cats to interrupt transmission; animals that are sources of meat such as pigs or sheep; and humans).

Models using outbred mice challenged perorally, parenterally and congenitally are available. Mice and rats with specific immuno-deficiencies have also been studied. Pig and monkey models have been used; studies have been performed with cats and sheep.

4.1.2 Future studies in animals

(i) Mice

It is important to work with immuno-competent mice other than for cell transfer studies or studies to define potentially protective immunological mechanisms.

Outbred mice are a reasonable starting point. Studies of either outbred or inbred mice may not be relevant to protection of other hosts.

Outbred and inbred mice from different suppliers may vary in susceptibility with respect to concomitant infections, etc. It would be helpful to be able to deal with this and thus reconcile data among laboratories, but no ready solution has been identified.

Peroral and parenteral immunizations are both potentially useful; congenital, peroral and parenteral routes are also pertinent for challenge.

Definition of end-points for protection is critical and could include:

- survival;
- cyst formation;
- tissue infection (including brain, muscle, cardiac and skeletal, liver, lung, spleen, amniotic fluid, placenta);
- recrudescence infection;
- antibody or CMI-produced; polymorphonuclear leucocyte activity;
- sub-class of antibody produced.

SCID-hu mice were considered useful as an immuno-compromised host model and as a type of prolonged culture system for human lymphocytes in the mouse peritoneal cavity.

SCID-hu mice have B and T lymphocytes under the kidney capsule but relatively few elsewhere. They are costly, cause difficulties in defining whether response is human/mouse, as well as in Class I and Class II mouse/human interactions. Primary immunization has not been possible so far against any infection using this system.

Human MHC transgenic mice may prove to be interesting as a model for protection studies and to define pertinence of MHC haplotype to protection in humans.

(ii) Rats

Nude rats with cell transfer and immuno-competent rats have been used for protection studies. A congenital model has been defined. Rats are more resistant than mice to parenteral infection with RH but are apparently very susceptible to peroral infection with oocysts.

(iii) Pigs

Pig meat is a source of transmission to humans. Elimination of T. gondii cysts from pig meat is being studied. The RH strain of T. gondii does not persist in pigs and could thus be used as a vaccine. It confers immunity to peroral re-challenge with oocysts and cysts, measured as survival and as protection against cyst formation. Genetically-defined miniature pigs are being studied, specifically, for their susceptibility to T. gondii tachyzoites, oocyst and cyst challenge, and their humoral and cell-mediated responses to T. gondii. It has yet to be clarified whether they will be similar to human immune responses.

(iv) Cats

Studies of protection in cats should be performed, although immunization of all cats is not feasible. Current T. gondii strains that do not complete the entero-epithelial cycle may provide information concerning where this cycle might be blocked immunologically or pharmacologically. Isolation of a strain of T. gondii (T263) that does not produce oocysts when given to cats is of interest as a potential vaccine.

(v) Sheep, cows

These are most pertinent to study vaccination of meat animals rather than as models for vaccines to protect humans. The S48 strain of T. gondii is marketed in New Zealand and the Netherlands as a vaccine for sheep. It does not form cysts; the degree and duration of protection need to be further characterized.

(vi) Monkeys

Rhesus monkeys are being used in vaccine studies in the Netherlands. Such studies should be reserved for the most advanced and promising preparations after initial testing in mice. They are very costly.

(vii) Humans

Vaccines for humans are far off and most difficult. Recent summaries of the history of development of polio virus, rubella, varicella, adenovirus, influenza virus and Hemophilus influenza vaccines have been reviewed. The general paradigm for vaccine development appears to have been development of attenuated vaccine strains or preparations, study of protection in mouse models and then, in some instances, in Simian models. There has sometimes been study of whether antibody or CMI is produced, associated with resistance usually measured as survival. Trials in humans to study antibody production and rates of protection then follow. It appears that current safety guidelines for vaccine development are more stringent. The approach to vaccine development involves testing in tissue culture when possible, then in mice, then non-human primates, then in humans in Phase 1 clinical trials, and so on. Human vaccination requires a different level of concern about safety. Phase 1 studies of vaccines for humans would probably involve the determination of antibody and CMI responses in members of the normal population. Pregnant women would be the last to be tested.

4.2 Challenge strains

4.2.1 General considerations and current status. It has previously been assumed that all strains of T. gondii are roughly equal, although demonstrating a wide range of virulence in the mouse model. A recent molecular analysis of strains using RFLP (restriction fragment length polymorphism) markers has been able to add considerable insight into the genetic make-up of T. gondii strains.

Among the probes used for RFLP analysis, both p30 (SAG1) and PEF (penetration enhancing factor) (ROP1) and several randomly isolated cDNA's have demonstrated a perfect correlation with virulence. When examined with a total of 11 markers (on 8 different chromosomes), the genotypes of all virulent isolates were so similar as to suggest they are in fact clonal. A similar correlation between virulence and genotype has also been reported, using iso-enzyme analysis and by using a monoclonal antibody recognizing an antigen correlated with virulence. It may be emphasized that all three of these approaches (RFLP, iso-enzyme, and monoclonal antibodies) demonstrate a correlation with virulence but do not establish that these genes or their products are directly involved in virulence.

4.2.2 Future considerations. The relatedness of virulence of a strain of T. gondii in mice and virulence in humans is felt to be an important area for future study. It may be reasonable that vaccine trials in animals include a number of avirulent and virulent strains. It may be reasonable that strains that complete the full life cycle be utilized. They should not be mixed genetically. Care should be taken to prevent increased virulence of strains. Parasite banks should be passaged in a manner that retains the strains as closely as possible to this initial virulence. Consideration of stage of challenge (sporozoite, versus bradyzoite versus tachyzoite) is also important.

4.3 Levels and types of protection required

4.3.1 General considerations and current status. There are numerous studies which demonstrate the importance of antibody CMI (CD4, CD8, cytolytic, and activated macrophages) in immunity. However, it remains to be defined which immune responses are necessary and sufficient for protection. This may well differ for different species and the form of infection for which protection is desired (e.g. peroral, cellular invasion, initial acquisition, congenital, oocyst shedding).

4.3.2 Future considerations. Antibody alone can protect when protection is defined as enhanced survival of, say, rodents. However, it remains to be determined how the earliest development of immune systems would protect humans. Some information could be derived from (1) chronically infected immuno-compromised women who have transmitted infection to their babies when they have antibody and from (2) women who have passively provided antibody to their infants infected during birth and who may not have had antibody to epitopes critical for protection, although they do have some measurable antibody.

As many sub-classes of immunoglobulin as possible should be tested. The role of secretory IgA in protection needs to be further defined. Protective responses in the cat intestine may be different from human intestine and congenitally-infected infants.

4.4 Genetics

Initial acquisition, survival, parasitemia, host cellular (e.g. suppressor T cells) and antibody responses, containment of chronic infection may all be influenced differently by host genes.

Outbred mice are a reasonable way to start studies, but genetic restriction may markedly affect response to vaccines and susceptibility. The relative pathogenicity of T. gondii strains may also be influenced by the genotype of the parasite. It may be possible to identify genotypes that are associated with virulence in the acute or chronic stage of infection (above) or genotypes associated with strains of T. gondii that cause pathological lesions during chronic infection versus those that do not.

T. gondii strain is also a critical factor in development of inflammatory response in chronic toxoplasmic encephalitis.

5. PLANNING AND MANAGEMENT OF FIELD VACCINATION TRIALS

Strategy

- (1) Collection of epidemiological data for sheep, with special emphasis on abortion.
- (2) Development of epidemiological and mathematical models for toxoplasmosis/T. gondii infections in sheep.
- (3) Adaptation of these models to the situation in humans.

There is a need for better and defined diagnostic methods. Field trials will be facilitated by the continuous development of sensitive, specific, defined and cheap diagnostic methods to obtain epidemiological data. Governments should address these problems in their funding programmes.

6. WORK PLANS FOR 1992-1994

- (1) Cloning of genes and characterization of enzymes, e.g. DNA polymerase, that might be useful targets for sub-unit vaccines.
- (2) Clone T cells from naturally-infected humans and determine the parasite polypeptides associated with the host-protective response.
- (3) Determine which T. gondii antigen(s) stimulate protective T cells in animal models.
- (4) Expression in BCG for vaccination experiments in mice, congenital toxoplasmosis in rats and in sheep.
- (5) Expression of peptide epitopes in a novel viral model MS2, immunogenicity of peptides in the model, evaluation of MS2 as an antigen presentation system.

- (6) Basic research on the cellular localization and functional role of T. gondii proteins, with regard to their suitability as target antigens for vaccination.
- (7) Stage-specific expression of antigens and the potential release of molecules from encysted parasites and bradyzoites.
- (8) Production of recombinant proteins (GRA1, GRA2 and GRA5) to improve standardization of diagnostic methods using recombinant peptide fragments because diagnosis is essential for epidemiology.
- (9) Collection of epidemiological data of T. gondii infections in sheep and in cats, with special emphasis on humoral responses to recombinant antigens that may be targets for recombinant vaccines.
- (10) Development of an epidemiological model for relationships between prevalence of T. gondii infection and frequency of abortion in sheep.

7. CONCLUSIONS AND RECOMMENDATIONS

7.1 Conclusions

The group agreed that toxoplasmosis research in vaccine development should be strengthened in conformity with its public health and epidemiological significance in human and animal populations. The group should take international initiatives to improve all aspects of present knowledge of the arts of vaccinology and immunology on toxoplasmosis, in line with plans of work of the group in 1992-1994.

Live vaccines are not suitable for use in humans.

7.2 Recommendations

- (1) Biosafety aspects in human and animal populations should be explored in the process of candidate vaccine development.
- (2) The search for new candidate antigens should continue.
- (3) Studies on the presentation of antigens should be continued.
- (4) More should be known about the cell-biology and molecular biology of T. gondii.
- (5) Better and well-defined diagnostic tests are needed to explore the epidemiology of T. gondii.

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ANNEX I

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