Round Table I
Immunization and Treatment

Convenors:  M. YOSHIOKA, Tokyo, AND P. WHITTLESTONE, Cambridge

Contributors:  H. MIZUTANI, Tokyo: M. pneumoniae: Immune Mechanisms
W. MASIGA, Kenya: M. mycoides subsp. mycoides Vaccination
C. HOWARD, Compton: M. dispar and M. bovis Vaccination
I. NONOMURA, Gifu: M. synoviae Immunization
R. ROSS, Ames: M. hyopneumoniae Immunization
E. HAYATSU AND M. YOSHIOKA, Tokyo: M. pneumoniae Immunization
M. MURATA, Fukuyama: M. gallisepticum: Treatment with Antibiotics

The invited contributors to the immunization section of the round table made the following main points.

Dr. Mizutani stressed that although both specific antibodies and cellular immune responses develop in the course of infections, the significance of the different responses varies widely according to the particular infection. He and colleagues had previously suggested that in pneumonia caused by Mycoplasma pneumoniae there is a relationship between the degree of the cellular immune responses and the pulmonary infiltrations which develop during the course of the disease. Recently their studies had revealed, by means of the ELISA test, the presence of rheumatoid factor (RF) in the sera of patients with M. pneumoniae infection. RF was present frequently in patients, and individual patients' RF levels fluctuated according to the clinical stage of the illness, which suggested that RF was related to the immune response to M. pneumoniae infection.

Dr. Masiga traced the development of vaccines for contagious bovine pleuropneumonia from 1850 to the present day. The earliest vaccines were infectious pleural fluid or lymph inoculated subcutaneously, which produced severe reactions—albeit less damaging than the natural disease. This type of vaccine was replaced in the 1920s and 1930s by broth cultures of various attenuated M. mycoides strains, and, more recently, freeze-dried broth vaccines had become available in Africa. It is generally considered that the passage level of the vaccine is important, cultures at less than 16 passages being too virulent and those above 73 passages being non-immunogenic. Although there have been attempts to develop inactivated vaccines, the resultant immunity has been generally disappointing. The mechanisms of protection against M. mycoides infection are not well understood. There appears to be no relationship between the levels of circulating antibody as measured by comple-
ment-fixation and growth-inhibition tests. Nevertheless work by Dr. Masiga and colleagues had shown that transfer of serum did convey some protection to the recipient cattle. There is also some suggestion that cell-mediated immune responses play a role in resistance to infection.

Dr. Howard described experiments to immunize calves against respiratory infection with either *M. bovis* or *M. dispar* strains by injecting killed organisms by various routes. Protection was assessed, after challenge, by making counts of the numbers of mycoplasmas in the lungs of the vaccinated calves compared with unprotected control calves. The intratracheal inoculations consisted of antigen in saline; the intramuscular and subcutaneous inoculations were antigen mixed with Freund's complete or incomplete adjuvant. With *M. bovis*, two inoculations given intramuscularly or two inoculations intratracheally did not result in any protection, whereas protection did follow either intramuscular inoculation followed by intratracheal inoculation or three subcutaneous inoculations. Immunity appeared to be related to antibody in lung washings at the time of challenge, but not to the circulating antibody level. With *M. dispar* three subcutaneous injections generated a low level of protection, whereas no immunity occurred after intramuscular followed by intratracheal inoculation. Thus *M. dispar* appeared to be less immunogenic than *M. bovis* in young calves.

Dr. Nonomura had studied the capacity of a temperature-sensitive mutant of *M. synoviae* to protect chickens against air sacculitis induced with this mycoplasma. This mutant (MSts44) had been selected by treating the wild strain (1-3SN) with N-methyl-N'-nitro-N-nitroso guanidine. The mutant would not grow in broth at 39.5°C and was found to be non-pathogenic in bursectomized chickens. The mutant was stable after five passages through chickens. The intranasal inoculation of MSts44 in doses in excess of $10^{11}$ colony-forming units was found to protect chickens against experimentally induced air sacculitis for at least 21 weeks. Before it is known whether MSts44 can be used as a vaccine, field trials will be needed.

Dr. Ross first reviewed the earlier evidence indicating that vaccination against enzootic pneumonia of pigs caused by *M. hyopneumoniae* might be feasible, since experimental vaccines had afforded some protection against gross pneumonia and mycoplasma colonization of the lung. In field trials the vaccines appeared to fail completely. The author and colleagues had prepared vaccines from strains J and 11 of *M. hyopneumoniae* killed with 0.15 percent formalin. In one experiment $10^9$, $10^{10}$, or $10^{11}$ organisms each reduced the extent of pneumonia on subsequent challenge. In another experiment some protection followed either two intramuscular injections or one intramuscular followed by one intratracheal inoculation.

Dr. Hayatsu reported on work in collaboration with Dr. Yoshioka and reviewed the evidence for the protective effect of various immunogens against *M. pneumoniae* infection. First, it had been shown that the administration of inactivated vaccine to hamsters lowered the frequency of experimentally induced *M. pneumoniae* pneumonia, but in field trials in man such vaccines were not very effective (< 67 percent). Live attenuated vaccines had induced significant protection in both hamsters and human volunteers, but unfortunately such vaccines were not sufficiently attenuated for general use in man. More recently it had been shown that the vaccination of hamsters with protein and polysaccharide extracted from *M. pneumoniae* cells conferred a significant resistance to challenge.
In Dr. Yoshioka's own experimental study, three doses of an inactivated adjuvant *M. pneumoniae* vaccine protected hamsters significantly against challenge and reduced the level of mycoplasma colonization in the respiratory tract. No mycoplasmas could be isolated from hamsters that received six doses of vaccine before challenge. The protective effect persisted for at least six months after the last vaccination and there was some protection up to ten months. There was a correlation between the resistance to infection and serum antibody titers, especially those measured by CF. Bronchial washings from hamsters that had received three vaccinations contained a low level of MI antibody. The inoculation of hyperimmune rabbit serum into hamsters conferred protection against mycoplasma colonization and MI antibody was detected in bronchial washings. The immune mechanisms against *M. pneumoniae* are still unclear, and much research is needed to develop and evaluate vaccines for use in man.

In the section of the round table concerned with drugs, Dr. Murata reviewed the published data on the prevention and treatment of *M. gallisepticum* infection in chickens with antibiotics, and related this evidence to the recent work of himself and his colleagues. The minimal inhibitory concentrations of a range of antibiotics against 85 strains of *M. gallisepticum in vitro* have been determined and surveys also made on the development of drug resistance. The prophylactic or therapeutic effect of spectinomycin, doxycycline, linco-spectin, cephalexin, josamycin, and tylosin have been tested *in vivo* using chicks.

The round table discussion concentrated on the problems of immunization against mycoplasmal disease, especially respiratory disease in man and animals. In introducing the speakers, Dr. Whittlestone said that the great economic importance of mycoplasma diseases in animals and the absence of effective drug regimes for the control of the diseases emphasized the importance of work leading to the eventual development of good vaccines. Currently vaccines are available for very few diseases and even with the well-known vaccines for contagious bovine pleuropneumonia and related diseases further improvements are desirable. One of the basic problems stressed by Dr. Yoshioka was that in natural infections, pathogenic mycoplasmas remain in the host for a long period, so that it is clear that even the full range of immunological reactions to the organism do not easily exclude the mycoplasmas from the body. On the other hand, once animals have eventually recovered they are strongly immune to re-infection. The immunity developing following the use of inactivated vaccines has been disappointingly low, even when antigen had been inoculated repeatedly and in combination with adjuvants.

There was discussion on the high cost of inactivated vaccines because of the expense of the volume of media needed to grow the whole antigen dose. With the continuous development of better media which would grow mycoplasmas to high titers, this problem would decrease, at least for some mycoplasmas. It was clear that very little information is available on the requirements for the maximal stimulation of the immune system; more data are needed on for, for example, antigen dose, best routes of inoculation, intervals between doses, numbers of doses, and particularly which adjuvants are both effective and acceptable. Speakers stressed the need for more fundamental information on the immune responses to mycoplasmas, so that these could be measured to assess the effectiveness of the immune response to different experimental vaccines and vaccine regimes. Although, finally, field trials to assess actual protection would be needed, understanding and measurement of the immune responses would enable vaccines to be developed more logically and economically.
The hope was expressed that the recent development of techniques to isolate mycoplasma antigens would lead to the identification and manipulation of those antigens concerned with stimulating the host immune system.

Dr. Barile asked about the different classes of antibody developing in response to the various vaccine schedules. Dr. Howard replied that in calves previously inoculated with antigen intramuscularly he could detect very little antibody in the respiratory tract, but he had detected some IgG₁ and IgG₂. When intramuscular injection was followed by intratracheal inoculation of antigen, more antibody could be detected, which was mainly IgG₁ and IgG₂ but there was also some IgA, which was specific for the inoculated mycoplasma. Dr. Howard stressed that the responses could be different in different species; thus the types of immunoglobulins detected in the respiratory tract of man were not identical with those detected in the calf's respiratory tract. Dr. Yoshioka emphasized the poor correlation generally found between measurable circulating antibody and recovery from mycoplasmal disease, yet passively administered hyperimmune serum often protected hosts well against experimental infection. He wondered whether there was any explanation for this apparent contradiction.

There was discussion on the inadequacies of the experimental inactivated vaccines so far tested: even the best vaccines have given only partial protection in that they have only resulted in a reduction of the extent of pneumonia and a lower level of mycoplasma colonization (when vaccines were compared with unvaccinated controls post-challenge).

Dr. Tully suggested that the selection of a mycoplasma at low passage level might provide a more immunogenic antigen, since antigen concerned with stimulating protective mechanisms in the host might well be lost during passage in vitro. He also thought that growing the mycoplasma in tissue culture rather than in broth would be worth trying.

Dr. Cassell enquired whether there had been any study in domestic animals comparing the degrees of protection developing in the upper respiratory tract (nasal turbinates) compared with the lower tract (trachea and lung). Dr. Howard replied that in the calf it was easier to protect against lower tract infection with a mycoplasma than against nasal infection.

Dr. Fernald recalled that in the literature there were fragments of evidence that sometimes vaccines might actually result in increased susceptibility rather than protection, and enquired whether there was any additional information. Dr. Ross said there was one report from Switzerland of an enzootic pneumonia vaccine trial in which vaccinated and non-vaccinated pigs were moved into an infected environment; more extensive lesions of enzootic pneumonia developed in the vaccinated pigs. In a repeat experiment this did not occur.

The possibility of attenuated vaccinal strains reverting to virulence was raised by Dr. Jordan. With regard to the live *M. mycoides* vaccines which had been extensively used in the field, Dr. Masiga said that the risk of reversion must be very small because of the virtual absence of any positive evidence; there was, however, one report that the attenuated T₁ strain regained its pathogenicity after five serial passages in cattle. Dr. Nonomura said there was no information on the possible reversion
of the Ts mutant of *M. synoviae*. Dr. Jordan also enquired whether there was any evidence of enhanced pathogenicity of the Ts mutant of *M. synoviae* if it was used concurrently with other vaccines, e.g., infectious bronchitis or Newcastle disease virus. Dr. Nonomura said there was no evidence on this point. In reply to a question from Dr. Jordan, Dr. Nonomura said that the *M. synoviae* vaccine protected against synovitis as well as against respiratory disease (during the round table on arthritis, additional evidence from other species supporting this general thesis was presented).

In conclusion, Dr. Whittlestone expressed the view that because of the many difficulties in developing effective inactivated vaccines for mycoplasmal respiratory disease and because conventionally attenuated vaccines were generally ineffective, new approaches should be encouraged; the development of Ts mutant vaccines might be the eventual solution in some diseases, since these vaccines would be expected to stimulate the defense systems of the respiratory tract, and the cost of commercial production could be low enough for such vaccines to be cost-effective.