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Some unanswered questions about vaccination

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I pondered for several months over what I should write for this 100th Volume of *FEMS Microbiology Letters* before deciding that it might be useful to pose some questions about vaccination for which there seem to be no adequate answers. Although I have put these questions verbally to colleagues over several years, I thought that by putting the same questions in writing, it would not only help to clarify my own thoughts, but it might even bring some answers. To avoid accusations that I may have become too philosophical and impractical, I have also focussed on one of these problems, namely antigenic variation, which I think could be overcome within the foreseeable future as we learn more about the structure of epitopes which evoke protective immune responses.

The questions that intrigue me are:

- (1) Why do we only get measles once, but are constantly getting colds or influenza? Antigenic variation is the usual answer to the second part of the question. But the question I am asking is why there is antigenic variation with some agents but not with others.

- (2) When there is no antigenic variation, why do we remain immune for life? Is it constant re-stimulation with the same agent or a relevant epitope? Or is the agent constantly ticking over in our bodies, gently tickling our immune system?
- (3) Why do some of us constantly get herpes? It is known that the virus 'hides' in the nerve cells between episodes, but is there antigenic variation here too?
- (4) Are live vaccines actually better than inactivated vaccines? The immunity evoked by a live vaccine will clearly be more like that produced by infection with the 'natural' agent. But there is the example of the study by Bottiger [1] which showed that even 12 years after the last inoculation of inactivated polio vaccine the neutralizing antibodies in the 18-year-old people being studied were still at a high level. Similarly, Simonsen and his colleagues [2] have concluded that vaccination against tetanus, which consisted of primary vaccination in infancy and one re-vaccination five years later, secures continuous protection to about the age of 25. Clearly it is not always necessary to use a live vaccine to evoke long-term immunological memory.

One question in particular which has intrigued

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me is that of antigenic variation. This variation in microorganisms is the cause of many problems, not only in the general population but also for public health authorities who seek to protect us by advocating or even insisting on vaccination and for manufacturers of vaccines who must keep ahead of the game if their products are to be useful. The examples that spring to mind are influenza and the common cold in man, and foot-and-mouth disease and bluetongue in domesticated livestock. The epidemiology of the common cold is complex because colds can also be caused by adenoviruses and coronaviruses although most are caused by rhinoviruses. Moreover, the essentially trivial nature of the common cold means that little or no effort goes into identifying the agents causing the illness.

Even with those diseases which have a single causal agent, however, there are problems of heterogeneity which, although suspected for many years, can only now be studied because of the development and application of new molecular techniques. The first step in the analysis of the composition of natural populations of microorganisms is usually their cultivation in the laboratory. With viruses which are to be used for preparing vaccines, this procedure can, and often does, lead to the selection of a single component of the original population. With influenza virus, for example, Schild et al. [3] showed that cultivation in eggs (which is the conventional substrate for vaccine production) selected sub-populations which differed antigenically from virus from the same source which had been grown in mammalian cell cultures.

Similar observations have been made with foot-and-mouth disease virus. Dinter, Cowan, Martinsen, McVicar and their colleagues [4–7] demonstrated more than 20 years ago that by growing the virus in tissue culture, antigenic variants could be selected which were neutralized much less well than the parent virus by antiserum against the parent virus. This selection occurred with viruses belonging to serotypes A, O and Asia 1, and presumably occurs with the other serotypes. Moreover, passage of viruses belonging to serotypes Asia 1 and A in baby hamster kidney monolayer cells gave products which were antigenically

distinct from the same virus grown in suspended cultures of the same cells [8–10].

One example which has been studied in detail pinpoints the dangers inherent in such procedures. Virus isolated from an outbreak of the disease in the UK in 1932 was for many years a 'model' virus of serotype A for research work on FMD at the Pirbright Institute in Surrey. When the Plum Island Animal Disease Center in the USA was opened in 1953, samples of tongue epithelium from a steer infected with this virus were provided by the Pirbright Institute, and a considerable part of the initial research at Plum Island was done using this material as a starting point.

Even when using what would now be regarded as insensitive, old-fashioned serological techniques, it soon became clear that the virus contained at least two and probably three antigenic variants [5]. What was not realized at the time was the extent of the variation. This only came to light during a study by my group at Pirbright of the immunogenic structure of the virus [11]. A series of studies had shown that the major site occurred within amino acids 141–160 of VP1, the most exposed of the four proteins comprising the virus capsid. Subsequent x-ray crystallography has shown that this sequence occurs within a highly exposed region of the particle [12]. Peptides corresponding to this region on viruses belonging to different serotypes were shown to be highly immunogenic, eliciting good levels of neutralizing antibody, but with one exception. The antibody elicited by the peptide corresponding to the 141–160 region of VP1 of the model serotype A virus referred to earlier neutralized only poorly the virus which had been passaged once in baby hamster kidney cells using tongue epithelium from an infected steer. The sequence of the virus RNA had been determined in a collaborative study by the Plum Island group and a group from Genentech. Our immediate reaction at Pirbright was that the peptide had been synthesized incorrectly, or the nucleic acid sequence was wrong. In fact, neither of these suppositions was correct. The problem was far more interesting. A sample of the Plum Island virus, which had been used to provide the RNA for the sequencing studies, was

neutralized extremely well by the anti-peptide serum.

In the meantime, sequencing the RNA coding for the 141–160 region of the Pirbright virus was causing problems at the positions coding for amino acids 148 and 153. However, by picking ten plaques and sequencing the RNA from their harvests, we showed that three had a Ser 148 Leu 153 combination, five had Leu 148 Pro 153 and two had Ser 148 Ser 153. Since the Plum Island virus was Phe 148 Pro 153, we then knew that there were at least four chemically distinct variants. Moreover, the viruses could be readily distinguished by serological tests. Subsequent circular dichroism spectroscopy studies have shown that the structures of the four peptides are different [13]. It should be added that the sequences of the entire capsid protein region of the four viruses are identical except at positions 148 and 153 (B.E. Clarke, personal communication).

For a variety of reasons the study had to be discontinued until I resumed work on the virus at Plum Island in 1990. It had been shown in the meantime by the group at Plum Island [14] that the variants identified by Cowan [5] also differed in sequence at 148 and 153. Moreover, two variants isolated by monoclonal antibody pressure also differed (Phe 148 Ser 153 and Phe 148 Gln 153) at the same two positions [15]. But to make matters even more complex, we have now obtained evidence, by direct cloning and sequencing of the RNA from virus-infected tongue epithelium provided to Plum Island by the Pirbright Institute in 1958, that there are at least two more variants differing at the same two positions. The total number of variants positively identified now stands at eleven.

The message for vaccine manufacturers is clear. The virus causing an outbreak could be a mixture of antigenic variants whose variability is significant in terms of protection. Consequently, the virus to be used for a vaccine should be as closely related antigenically to the outbreak strain as possible. How can this be ensured? From a practical point of view the best way is to determine whether the antiserum produced by the vaccine neutralizes the outbreak strain, ideally as well as it neutralizes the virus from which the vaccine

was produced. However, the need to 'adapt' viruses from field outbreaks so that they grow in high enough yields for vaccine production can mean that selection of the fastest growing strain takes place. This may not be critical antigenically but it remains a possibility that the cultured organism represents only small proportion of the diverse population in the initial isolate.

To identify a problem is one thing, but to solve it is another. What should we be aiming at to meet the problem of antigenic variation? Can we design vaccines which would have broader antigenic spectra? Our present structural studies with FMDV have this objective. By combining the spectroscopic analysis of the loop region containing the immunogenic site with serological data, we hope to determine the chemical and structural basis for antigenic variation. The broad spectrum sequence which may emerge could then be presented to the host either as a synthetic peptide or even as a hybrid virus, created by using the recombinant viruses which have now been described (ref. 16 and P.W. Mason, personal communication).

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