Virus structure

Crystallography attacks the cold

from Don C. Wiley

A LITTLE under eight years ago Harrison and co-workers published[1] the first three-dimensional structure of a virus after ten years of effort. Now it has taken Rossmann *et al.* the incredibly short period of thirteen months to determine the three dimensional structure of a rhinovirus — the causative agent of the common cold. Their achievement, reported on page 145 of this issue[2], is the result of a *tour de force* of modern X-ray crystallography, which is entering a new era. It is certainly nothing to sneeze at.

Perhaps the most intriguing discovery to emerge from the structure is that the tertiary folds and quaternary organization of the polypeptides in rhinovirus are remarkably similar to those found in tomato bushy stunt virus[1] and southern bean mosaic[3] virus, both icosahedral plant viruses, indicating an evolutionary relationship between these infectious agents. It will be particularly interesting to compare the structure with that of poliovirus, which is soon to be published by Hogle and co-workers[4]. This will be the subject of a future contribution to these columns.

But will knowledge of the structure lead to a cure for the common cold? The main reason that we do not develop immunity to colds, the way we do to childhood diseases like measles, and also the reason that no one has developed an effective vaccine against colds, seems to be that rhinoviruses exist in over eighty antigenically distinct serotypes (poliovirus, by contrast, has three). And each is different enough to escape antibodies against the other, but all are capable of delivering the same nasty cold. Through a collaboration with Rueckert's laboratory, Rossmann *et al.* describe four immunogenic sites on the virus that can generate neutralizing monoclonal antibodies in mice. The prospect of developing a 'comprehensive vaccine' against these sites seems remote, however, because the evidence indicates, presumably because of their exposed positions on external loops, that they can accept changes in structure (sequence) without consequent disruptions of any viral functions. So they may be able to stay ahead of the vaccine designers as well as our immune systems.

May there, instead, be a conserved epitope, shared by all rhinoviruses that could be a vaccine target? One prospect might have been the receptor-binding cavity, which binds the virus to a receptor on a target cell. Unfortunately, it seems to be in a deep cleft inaccessible to antibodies. A similar situation has been observed for the two glycoprotein surface antigens of influenza virus[5,6], where the conserved residues making up the receptor-binding pockets are either inaccessible to antibodies, or surrounded by a rim of amino acids that can vary and in so doing dislodge antibodies targeted at the conserved site. It remains to be seen whether rhinovirus carries some other common, conserved epitope that would not change structure under the selective pressure of antisera and against which neutralizing antibodies could be artificially stimulated (by a peptide?). In the meantime, as with the crystallographic studies of other infectious agents[5,7], basic issues such as the structural nature of immunogenic regions and the mechanism of immunological neutralization can now be studied in atomic detail.

Macromolecular crystallography is often misunderstood. It sometimes appears to be just a descriptive anatomy of macromolecules, providing complicated illustrations for textbooks. At its best, however, it provides the starting point for detailed research into how large molecules work. The three-dimensional patterns of chemical reactiveness on a macromolecule are the key to chemical analysis of the capabilities of that molecule. For example, one can now foresee three non-immunological approaches to curing the common cold. First, knowing the structure of the receptor-binding site on the virus should stimulate efforts to design a tight-binding specific inhibitor of virus–cell attachment. Second, structural

Sir Frank MacFarlane Burnet (1899–1985)

MACFARLANE Burnet, joint winner of the 1960 Nobel Prize for Physiology and Medicine and originator of the clonal selection theory, which forms the basis of modern immunology, died peacefully on 31st August, 1985. In this technological era, we can ask whether anyone will ever sweep as broadly or dare as boldly as the man who dubbed himself "the last of the great amateurs". The essence of Burnet's creativity was his ability to move from the restricted, reductionistic framework of particular experiments to nature's grand design, always searching for the general biological principle.

Burnet's first interest was in bacteriophages and he was the first to realize that the hereditary phage particle integrates itself into the 'hereditary constitution' of the lysogenic bacteria as a non-infectious anlage which multiplies in step with the bacterial cell. Animal viruses, much harder to grow than phages, preoccupied Burnet for the next thirty years. He improved the techniques for growing viruses in chick embryo, established the chief quantitative method for studying viral replication and isolated the causative organism of Q fever — a rickettsia and not a virus.

Burnet's interest in immunology was a natural outgrowth of his broad approach to virology. Three observations intrigued him particularly. First, chick embryos could not be immunized, no matter how hard he tried. Secondly, mice infected *in utero* with lymphocytic choriomeningitis virus had been shown by Traub to carry virus in their blood and tissues for life, without forming antibody. Thirdly, Burnet was struck by Owen's observation that dizygotic twin cattle which shared a common placenta could be born with and continue to carry two types of red blood cell, their own and that of the twin, without an immune response. Burnet predicted that an antigen artificially introduced into an immature immune system would lead to the animal being tricked into thinking that the antigen was not foreign. Billingham, Brent and Medawar provided the necessary experimental evidence to establish immunological tolerance as a reality and so Burnet and Medawar shared the Nobel prize for the discovery.

Burnet's most profound and unequivocally correct theoretical insight came some years after all the excitement about tolerance. Stimulated by Jerne's 'natural selection' theory, Burnet postulated the clonal selection theory in 1957. The essence of this is that lymphocyte cells bear on their surface specific immunoglobin molecules reflecting the specificity of the antibody that will later by synthesized once the cell is activated. A process of "somatic mutation or conceivably other inheritable changes" leads to diversification such that each cell has only one specificity, but the population as a whole constitutes a repertoire "corresponding probably with varying degrees of precision to all, or virtually all, the antigenic determinants that occur in biological material". Antigen serves as a selective stimulus, causing preferential proliferation and differentiation of the clones that have receptors for that antigen. He saw self-tolerance as involving the deletion of clones with receptors for self antigens. The cellular and molecular vindication of this theory gave Burnet his most lasting satisfaction.

Burnet was a biologist. His love-hate affair with biochemistry, which led to a brief but damaging rejection of the worth of molecular biology (*Lancet* (i) 37; 1966), stemmed from his belief that biochemists should serve as superior servants to the biologists responsible for uncovering nature's real secrets. Despite this ambivalence, he sponsored some first-rate biochemical work. Above all he will be remembered as one who believed science is primarily about ideas. G. J. V. Nossal