HIV

Antibodies with a split personality

Spikes on the surface of HIV to which antibodies can bind are sparse. One of nature's solutions is to sometimes produce antibodies that bind tightly to a spike with one arm and grab another structure with the other arm. SEE LETTER P.591

ANDREAS PLÜCKTHUN

Antibodies are usually said to have two main features: highly specific recognition of their target antigenic determinant, or epitope, and the ability of each of their two arms to bind to a copy of the same epitope. For these characteristics to be effective, the target epitopes must be spaced closely together on the surface of the viral or bacterial pathogen. However, Mouquet *et al.*[1] argue on page 591 of this issue that, in the immune response to HIV, some antibodies are selected whose structure allows them to bind to the glycoprotein 'spikes' on the HIV surface with one arm, and to another, undefined, molecular structure with the other arm.

An HIV-1 spike is composed of two subunits: three molecules of the surface glycoprotein gp120 link non-covalently to three molecules of the transmembrane envelope glycoprotein gp41 (ref. 2). The external portions of these two subunits have been engineered in a form known as gp140 to isolate neutralizing antibodies that recognize either of the subunits.

But each HIV particle has only 14 ± 7 spikes[3] (even though 73 ± 25 spikes would fit, and can be obtained, in engineered simian immunodeficiency virus[3]). So, if the HIV spikes were totally randomly distributed, the expected average distance between nearest neighbouring spikes would be 23 nanometres (Fig. 1). Although there is evidence[3] that HIV spikes are sometimes found only 15 nm apart — which would seem almost close enough for bridging by an antibody — most of the spikes do not have such a close neighbour (Fig. 1).

It is reasonable to expect that several HIV spikes must interact with receptors on the target cell for the virus to bind efficiently and fuse with the target-cell membrane. Indeed, greater numbers of spikes per HIV particle are correlated with increased infectivity[4], and probably with fusion efficiency. The question thus arises as to whether HIV derives any advantage from having a low spike density, and whether this is one of the many factors that make it so difficult for the immune system to raise a broadly neutralizing response to the virus[5].

Previous work[4,6,7] has shown that bivalent engagement of the IgG antibody improves antibody-mediated HIV neutralization. Although an inter-spike distance of 15 nm is probably just within the wingspan of typical IgG molecules (10–20 nm), the location, spatial orientation and relative directionality of the epitope will determine whether such bivalent binding is geometrically possible (Fig. 1). Even then, compared with monovalent binding, the energetic gain from bivalent binding of IgG to HIV spikes can be rather modest, as it is proportional to epitope density on the viral surface[8] and dependent on geometry.

Given these circumstances, the immune system might take advantage of a feature that is normally seen as a liability rather than an asset — a lack of absolute antibody specificity. This, however, is not wholly unexpected. Biochemists know that absolute specificity is an unachievable goal, because the irregular, feature-rich surface of a protein will almost certainly contain at least a few patches that will allow interaction with more than one macromolecule, albeit with low affinity. Also, practitioners of antibody-detection methods are all too familiar with the phenomenon of 'nonspecific binding' in many commercial antibodies.

Several mechanisms have been proposed to explain how antibodies might acquire multiple specificities, and a few have been verified from structural studies. In one case[9], different loop conformations in the antibody structure create differently shaped cavities to each of which quite unrelated antigens can bind. Indeed, antigens themselves can take advantage of this strategy: gp120, for instance, uses loop flexibility to present decoy conformations to the immune system[5]. In another case[10], an antibody was engineered to have two distinct but spatially overlapping antigen-binding sites in its variable domains and could thus bind tightly to either of two antigens. In these two examples, however, the antibodies are not polyreactive; that is, they cannot react with a wide range of antigens, but rather show a few well-defined specificities. By contrast, Mouquet *et al.*[1] could not identify a common molecular trait in their polyreactive anti-HIV antibodies that would explain the molecules' polyreactivity.

The authors[1] analysed 134 unique anti-gp140 antibodies derived from the blood of six patients infected with HIV and 52 control, non-gp140-reactive antibodies. They found that, as part of the immune response to HIV infection, most of the antibodies generated are polyreactive, suggesting that one antibody arm binds with high affinity to gp140 and the other arm binds with low affinity to a secondary epitope on the surface of HIV. The production of such antibodies by the immune system should be useful, as it might overcome the problem of the HIV spikes being so few and far between.

The identity of the secondary epitope(s) to which the anti-HIV antibodies binds, along with gp140, remains unclear. Mouquet and colleagues speculate that the phospholipid bilayer of the viral envelope might provide this low-affinity epitope, but it could equally be another epitope within the same spike. It is difficult to evaluate this phenomenon quantitatively. For practical reasons, the authors defined polyreactivity as the ability of an antibody to bind to polyelectrolytes (single- and double-stranded DNA), to hydrophobic compounds outside their normal context (lipopolysaccharide) and to other proteins (insulin and KLH). Other workers[11] have used different panels of substances to evaluate polyreactivity. But the results might even be influenced by the method used to prevent the antibody from binding to the plastic surface of the assay microtitre plate. This in turn could affect

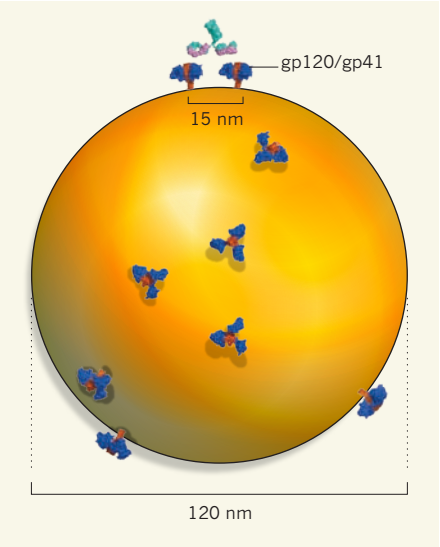

Figure 1 | Dealing with the problem of epitope shortage. The spikes on the surface of HIV are sparse and often far apart. This makes it difficult for the two arms of an anti-HIV antibody to bind to two epitopes on different spikes. Mouquet *et al.*[1] find that some anti-HIV antibodies are polyreactive. Such antibodies bind with high affinity to the epitope of a spike and may bind to another, as yet unidentified structure with low affinity. Here, the antibody, the spikes (only part of the structure of which is shown), the virus diameter and spike distribution on the virus are all drawn to scale, with the antibody drawn next to the pair that has the closest inter-spike distance (15 nm) found experimentally. (Graphic based on a figure prepared by Annemarie Honegger.)

which antibodies are classed as polyspecific.

Mouquet and co-workers tested in detail whether the binding strength of some of the bivalent anti-HIV antibodies to gp120 increased in the presence (compared with the absence) of excess KLH, as an indicator of simultaneous binding to two different epitopes, one by each arm. A monovalent antibody fragment prepared from the same antibody, and that can bind to only one epitope at a time, showed no enhancement. Rather, it showed the expected high affinity for gp120 and low affinity for KLH. However, this experiment does not directly reflect the situation at the viral surface, and so the authors also tested whether the anti-gp120 antibodies could bind to liposomes, which mimic the phospholipid bilayer of the HIV envelope. Compared with control antibodies, the polyspecific ones did indeed bind more efficiently to liposomes.

As the immune response develops, anti-gp140 antibodies undergo a high degree of mutation. To investigate whether such mutations influence the antibodies' polyreactivity, Mouquet *et al.*[1] reverted the mutations to the sequence present at the onset of the immune response in randomly selected anti-gp140 clones and analysed them. They found that most of the resulting clones significantly lost their affinity for gp140, but retained polyreactivity. This result suggests that the antibodies selected in the primary immune response to HIV have a higher tendency towards polyreactivity than the average antibody in the repertoire.

Is polyreactivity essential for antibodies to neutralize HIV? Little polyreactivity has been reported for two well-characterized, broadly neutralizing anti-gp120 antibodies — b12 and 2G12 (ref. 11) — or for more recently discovered anti-HIV antibodies[12] or the anti-gp41 antibody 2F5 (ref. 13). However, 2F5 has been described[11] as polyspecific, because it also recognizes the membrane phospholipid cardiolipin (which may also react nonspecifically with other proteins when it is not fully embedded in a membrane). Together, these data indicate that polyreactivity is not an essential feature of neutralizing anti-HIV antibodies.

Mouquet and co-workers' results[1] therefore suggest that the immune system may have the option of picking antibodies from the germline repertoire that have some degree of polyreactivity as a starting point to ensure the strongest eventual affinity. Binding to two different epitopes through polyreactivity might well contribute to functional affinity in some cases, and high affinity is known to correlate with virus neutralization. Alas, even with such naturally occurring polyreactive antibodies, HIV outsmarts the immune system, and so the prospect of a broadly effective vaccine based on broadly neutralizing antibodies remains elusive. ■

Andreas Plückthun *is in the Department of Biochemistry, University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland.*
e-mail: plueckthun@bioc.uzh.ch

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BIOGEOCHEMISTRY

Ocean biomes blended

The ratio of nutrient elements in marine subsurface waters is much the same everywhere, even though biogeochemically distinct ocean biomes exist. A modelling study that includes mixing solves this conundrum. SEE ARTICLE P.550

RAYMOND N. SAMBROTTO

The creation of organic matter at the ocean's surface by plankton, and its continuous rain to deeper waters, has a profound impact on most of the dissolved components of sea water. The main features of this marine biological pump have been clear for some time, as has its crucial role in slowing the atmospheric accumulation of carbon dioxide — it sequestrates more than 2×10^{15} grams of carbon derived from fossil fuels per year[1]. Quantitative predictions of how this system will interact with altered climate states are difficult to make, however, because of the broad physiological diversity of marine plankton, the absence of detailed information on the temporal and geographic variations of specific populations, and the lack of numerical tools with which to address the many variables relevant to defining biogeochemical fluxes.

On page 550 of this issue, Weber and Deutsch[2] use recent advances in numerical modelling to constrain major nutrient pools in the marine biological pump more effectively than ever before. In this way, they show that mixing between distinct ocean biomes has a significant role in producing marine biogeochemical relationships that were previously attributed to spatially invariant biological processes.

Laboratory and field studies document a wide range of nutrient utilization ratios — the ratios of carbon, nitrogen and phosphorus consumption — in marine phytoplankton, where the variation is driven by the organisms' taxonomic diversity and by responses of different populations of the same species to environmental conditions[3,4]. This information has coexisted somewhat uncomfortably with the dominant approach to modelling large-scale ocean biogeochemical fluxes, in which biogeochemical relationships among these nutrient elements are defined as constants, known as Redfield ratios[5].

There are good reasons for the long-standing use of Redfield ratios by marine biogeochemists, however. The primary one is the remarkable consistency of the relationships between the concentrations of major nutrients in subsurface waters, where most of the respiration of the organic matter originally produced by plankton occurs. These ratios (of carbon to nitrogen, or nitrogen to phosphorus, for example) are much the same as the average ratios found in plankton in surface waters[6]. Therefore, despite evidence disproving the existence of universal nutrient ratios in plankton, the empirical reliability of the Redfield ratios has made them useful in various applications. The demonstration by Weber and Deutsch[2] that horizontal mixing contributes significantly to maintaining the relationships among nutrients in subsurface waters suggests that the observed constancy of these ratios does not depend on a strict spatial uniformity in the vertical fluxes of nutrients, and instead is compatible with a range of surface biogeochemical regimes.

Weber and Deutsch's modelling work builds not only on relatively recent advances in ocean sampling, but also on advances in numerical modelling methods. As modern oceanographic sampling has pushed farther into remote (and nutrient-rich) regions such as the Southern Ocean, the existence of surface-water areas that have nitrogen/phosphorus (N/P) utilization ratios significantly lower than Redfield's has emerged[7,8]. Like terrestrial environments, these regions can be characterized as distinct biomes on the basis of temperature, mixing and light conditions[9]. In contrast to terrestrial systems, however, the fluid nature of marine biomes greatly increases the interactions between them, a feature that looms large in Weber and Deutsch's analysis[2].