A microbe becomes a pathogen by successfully evading the host’s immune responses, and the microbial strategies for so doing are legion. They include methods to avoid recognition by the immune system—for example, by antigenic variation as shown by influenza and HIV and by parasites or plasmodia, or by acquiring a host coat as is done by worms and retroviruses.

Another major mechanism is to avoid the effector mechanisms of the immune response. This can be done by subverting cytotoxic T cells by the production of decoy HLA molecules; or by subverting Fc function by producing Fc receptor homologues; or by subverting complement by producing homologues of complement control proteins (CCPs). Some viruses also have developed methods of subverting apoptosis in the cells that they infect.

This paper concentrates on the innate immune response. The definition of this term is a little fuzzy. Fearon and Locksley regard all mechanisms using germline coded molecules as being innate which therefore includes natural antibodies with germline V regions. Perhaps a more conventional definition is that innate mechanisms are those that are not specifically altered by prior exposure to the same pathogen.

In the first part of this paper some examples of subversion of the complement system will be described. The second half describes some much newer work from our laboratory that takes us into aspects of the innate immune response on mucosal surfaces that have not so far been described.

OVERVIEW OF THE COMPLEMENT SYSTEM

Figure 1 shows a greatly simplified view of complement activation by micro-organisms. Regulation occurs principally at two points—the first is action of the C3 converting enzymes and the second, action of the membrane attack complex (MAC).

Regulation of the C3 converting enzymes is produced by a number of proteins (fig 2), all of which are based on a single protein domain, the so-called CCPs or CCP domain, also called the short consensus repeat (SCR). These proteins are also all located mainly on the long arm of chromosome 1 at a single large locus.

Regulation of the MAC (fig 3) is brought about by a distinctly different protein, CD59. This protein is the only one of its kind in the complement system and similar proteins are not commonly found elsewhere, although its 3D structure (fig

**Abbreviations:** BSA, bovine serum albumin; CCP, complement control protein; GAS, group A streptococci; HEL, hen egg lysozyme; MAC, membrane attack complex; SCR, short consensus repeat; SIC, streptococcal inhibitor of complement; SLPI, secretory leucocyte protease inhibitor
4) does resemble some snake venoms in shape. It is GPI anchored to cell membranes, widely distributed, and important in protecting cells from complement mediated lysis.

**MECHANISMS OF COMPLEMENT EVASION BY VIRUSES**

The sources for this section are contained in the review by Lachmann and Davies.

Viruses have evolved a number of techniques for evading the complement system. One is the arrangement of epitopes in their surface proteins such that antibodies against them are not able to initiate complement fixation. The measles virus provides a good example of this strategy. Antibodies to the measles haemagglutinin, whether IgG or IgM, fail to activate complement by the classical pathway, presumably because the antigenic epitopes are so spaced that effective bridging cannot be obtained between them. This does not prevent measles virus infected cells (even in the absence of antibody) from activating the alternative pathway, but this is a relatively inefficient way of producing cell lysis.

Viruses (for example, HIV) have evolved mechanisms of entry into cells through complement receptors after fixing complement. This is analogous to the strategy used by the Dengue virus which can enter cells through Fc receptors after having bound antibody.

Viruses have also evolved means of acquiring host CCPs. Some viruses—for example, HIV and vaccinia, capture these proteins from the host cell membrane as they leave the cell. Other viruses cause the cell to synthesise specific molecules for this purpose. This in turn can be done in two different ways. One is by gene capture from the host. This will be discussed in relation to herpes virus saimiri. The other is by convergent evolution, where proteins with no structural similarity to host CCPs have evolved that complement control function. This is clearly seen in herpes simplex virus.

Herpes virus saimiri is a T lymphotropic tumour virus, which harmlessly infects New World primates but in Old World primates gives rise to tumours. Gene sequencing has identified two open reading frames encoding CCPs. One is a homologue of mammalian CCPs with particular homology to C4 binding protein. This protein is produced in two forms: an unspliced, transmembrane protein and a secreted protein produced by alternative splicing. Antibodies against both forms of this protein will neutralise the virus. More unusually, herpes virus saimiri also contains a homologue of CD59 which shows close similarity to human CD59 and, interestingly, an even greater similarity to squirrel monkey CD59. The squirrel monkey is the virus’s natural host and this suggests strongly that the viral CD59 gene has been captured from the squirrel monkey in relatively recent times.

When expressed in insect cells using a baculovirus system the herpes virus saimiri CD59 can be shown to have MAC inhibiting activity. In inhibiting lysis by human serum, viral CD59 is much less efficient than human CD59 but approaches
the activity of human CD59 when marmoset serum is used. On the other hand, when tested against lysis by rat serum it had the same activity as human serum; and using rabbit serum, where human CD59 is extremely inefficient, the viral CD59 gives a much stronger MAC inhibiting effect. These data are interesting in that they will assist in mapping the essential residues of CD59 that determine the specificity of reaction with different species of MAC.

Herpes simplex virus has a CCP—G-c1 in HSV1 and G-c2 in HSV2—which has no structural similarity to CCPs. G-c 1 and 2, however, do control C3 convertases, but their activity is slightly different from that of a typical mammalian CCP. Nevertheless, it is known that they are effective in vivo and that their absence reduces viral virulence.

CCPs also occur on a number of parasites. For example Trypanosoma cruzi has a molecule that has decay accelerating factor-like structures and controls C3 convertases. Schistosoma mansoni has a protein known as SCIP-1, which has CD59-like properties and shows some cross reactivity with antibodies to human CD59. SCIP-1 is made only in the mammalian host and not in the snail part of the parasite’s life cycle where resistance to mammalian complement is not needed. Bacteria do things rather differently. A number of bacteria bind the soluble mammalian CCPs, factor H, and C4 binding protein onto their surface as CCPs. These include Neisseria gonorrhoeae and Streptococcus pyogenes. However, in 1996, Åkesson and his colleagues described a novel protein which is secreted by M1 group A streptococci (GAS) and which did appear to act as a CCP. It is this protein that is the topic of the second half of this paper.

**STREPTOCOCCAL INHIBITOR OF COMPLEMENT (SIC)**

SIC is a 31 kDa extracellular protein with no obvious resemblance to mammalian CCPs and is secreted at high concentrations, about 5 mg/l. It has been shown to inhibit...
that these three components synergise in killing bacteria. SIC was found to be a C567 uptake inhibitor, and also to SLPI, but not to lactoferrin. The reaction of lysozyme is impressive in so far as the addition of SIC to lysozyme in solution causes their precipitation (table 1). SIC can be conveniently purified from streptococcal culture supernatants on lysozyme-Sepharose columns at 4°C (fig 5). It is eluted at about 0.3 M NaCl, showing that the binding is fairly strong at that temperature. SIC partially inhibits the lysozyme-induced killing of *Streptococcus suis*. The inhibition is not complete as *S suis* are exquisitely sensitive to lysozyme (fig 6). SIC inhibits the catalytic activity of lysozyme in vitro as measured by digestion of bacterial cell walls, the level of inhibition being up to 75%. The reaction is much more efficient at 4°C than it is at 37°C, where indeed it is weak (fig 7).

SLPI is a peptide of 11.7 kDa, widely distributed in mucous fluids. Its crystal structure is known and shows two similar domains. The C-terminal domain has protease inhibitory activity and the N-terminal domain has bactericidal activity. SLPI does kill the M1 strain of GAS, which lysozyme does not, and interestingly this activity is inhibited by SIC (fig 8). On the other hand, SIC has no effect on elastase inhibition by SLPI.

Competition experiments suggest that SLPI and lysozymes bind closely to each other on SIC (fig 9). However, we do not believe that the binding sites are identical because the properties of the binding are very different. The reaction of SLPI with SIC shows a slight paradoxical temperature dependence, occurring better at 37°C than 4°C. This shows that the binding is largely hydrophobic. The maximum number of SLPI molecules bound to each SIC molecule is probably two. On the other hand the binding of lysozyme is extremely temperature dependent and occurs effectively only at 4°C, showing the reaction to be largely ionic. The maximum number of molecules bound is probably four (table 2).

Thermodynamic calculations confirm that the reaction with SIC is largely entropy driven and has very small positive enthalpy, whereas the reaction with lysozyme has a very small entropy change and a substantial negative enthalpy. At 4°C the free energy changes are quite similar (table 3). These data

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**Table 2** Binding characteristics of SIC with SLPI and HEL. Reproduced, with permission, from ref 11. Copyright © 2002 American Society for Microbiology.

<table>
<thead>
<tr>
<th>SlpI $^*$</th>
<th>HEL $^+$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>K&lt;sub&gt;d&lt;/sub&gt; (µM)</strong></td>
<td>4°C</td>
</tr>
<tr>
<td>26.1 (4.1)</td>
<td>19.3 (3.4)</td>
</tr>
<tr>
<td><strong>No of molecules bound</strong></td>
<td>1.6 (0.1)</td>
</tr>
</tbody>
</table>

$^*$Results of three determinations performed in duplicate; $^+$Results of three determinations performed in triplicate.

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**Table 3** Physical chemical data—interaction of SIC with SLPI and HEL. Reproduced, with permission, from ref 11. Copyright © 2002 American Society for Microbiology.

<table>
<thead>
<tr>
<th>SIC HEL</th>
<th>K&lt;sub&gt;d&lt;/sub&gt; (µM)</th>
<th>Entropy (kJ/mol/K)</th>
<th>Entropy (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLPI HEL</td>
<td>6.4 (2.3)</td>
<td>0.035 (0.009)</td>
<td>26.4 (0.4)</td>
</tr>
</tbody>
</table>

Calculated from data in table 1.
suggest that the binding sites are different, although close to each other. They also suggest that the in vivo role of SIC is likely to lie predominantly in its binding with SLPI. SIC is, to the best of our knowledge, the first biological inhibitor of SLPI which has been described. It is clearly a target for developing antistreptococcal treatments. It is also likely to prove a useful reagent in probing in vivo activities of SLPI and lysozyme in both infective and uninfected models of mucosal inflammation.

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Authors’ affiliations
B Fernie-King, D J Seilly, A Davies, P J Lachmann, Microbial Immunology Group, Centre for Veterinary Science, University of Cambridge, Madingley Road, Cambridge, CB3 0ES, UK
Correspondence to: Professor Sir P L Lachmann; pjl1000@cam.ac.uk

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B Fernie-King, D J Seilly, A Davies and P J Lachmann

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