Ikawa, T., Kawamotot, H., Wright, L.Y.T., and Murre, C. (2004). Immunity *3*, 349–360.

Kee, B.L., and Murre, C. (1998). J. Exp. Med. *188*, 699–713.

Klinken, S.P., Alexander, W.S., and Adams, J.M. (1988). Cell *53*, 857–867.

Kondo, M., Scherer, D.C., Miyamoto, T., King, A.G., Akashi, K., Sugamura, K., and Weissman, I.L. (2000). Nature *407*, 383–386.

Nutt, S.L., Heavey, B., Rolink, A., and Busslinger, M. (1999). Nature *401*, 556–562.

Rolink, A.G., Nutt, S.L., Melchers, F., and Busslinger, M. (1999). Nature *401*, 603–606.

Singh, H. (1996). Curr. Opin. Immunol. *8*, 160–165.

Xie, H., Ye, M., Feng, R., and Graf, T. (2004). Cell *117*, this issue, 663–676.

Class II MHC: Sweetening the Peptide Only Diet?

MHC molecules typically bind peptides to create ligands for the T cell antigen receptor. In this issue of *Cell*, Cobb et al. (2004) report an unexpected association of class II MHC molecules with processed zwitterionic polysaccharides from pathogenic bacteria. The complexes appear to modulate the T cell dependent pathology of abscess formation.

MHC molecules and their relatives, the CD1 family, seem to adhere to something resembling an Atkins diet. Feasting on the products of protein and lipid processing, these molecules control T cell immune responses by binding peptides (MHC) or various lipid derivatives (CD1). These peptide and lipid ligands occupy a binding groove on MHC and CD1 proteins and present a composite surface for the T cell receptor to engage. Although a minority of MHC-associated peptides are glycopeptides, liaison of MHC with protein-free polysaccharide has not been observed. In this issue, Cobb et al. (2004) provide evidence that class II MHC molecules may relax their peptide only regime and form associations with unusual bacterial polysaccharides. In doing so they elicit disease-modulating T cell immune responses. For immunologists long used to the MHC/peptide paradigm, these novel data will be scrutinized with interest.

Today's report is the latest in a series of studies by Kasper and colleagues on T cell modulation of abscess formation following infection with bacteria such as the obligate anaerobe *Bacteroides fragilis* (Tzianabos and Kasper, 2002). Such organisms can be readily isolated from human abdominal infections and have been used in rodent models of intraabdominal sepsis and abscess formation. CD4 T cells activated by *B. fragilis* both contribute to abscess formation and can also protect when adoptively transferred intravenously prior to bacterial challenge (Tzianabos and Kasper, 2002). Unexpectedly, these protective T cells were activated by purified components of the bacterial polysaccharide capsule. This is surprising because the textbook view of immune responses to polysaccharide antigens is that they are T cell independent. Recent work from this group has identified the capsular structures that induce the CD4 T cell responses that modulate abscess formation. The T cell stimulating component from *B. fragilis* (called PS A2) turned out to be an unusual zwitterionic polysaccharide consisting of a pentasaccharide repeating unit each carrying a single positive and negative charge. Analogous zwitterionic polysaccharides (ZPSs) are found in the capsules of other bacteria such as *Streptococcus pneumoniae* and are also able to modulate abscess formation in a T cell-dependent manner. Although the repeating unit of different ZPSs is chemically distinct, the *B. fragilis* and *S. pneumoniae* structures solved to date both form right-handed helices with similar charge disposition (Wang et al., 2000; Choi et al., 2002). Moreover, interference with the zwitterionic character of the polysaccharide eliminates their T cell stimulating and abscess-modulating properties. Most recently, the T cell activating properties of ZPSs were shown to require antigen presenting cell (APC) /T cell contact and required class II MHC molecules on the APC (Kalka-Moll et al., 2002).

CD4 T cells are activated upon recognition of class II MHC molecules that have captured processed peptides within the endosomal/lysosomal compartments of antigen-presenting cells. Cobb et al. (2004) now show that ZPSs bind to class II MHC molecules and as for protein antigens, a functional endocytic pathway and ZPS catabolism are required before such an association can occur. Although enzymatic processing of ZPS is not yet ruled out the current data implicates chemical cleavage of the bacterial capsular ZPS by reactive oxygen and/or reactive nitrogen species. In an important experiment that appears to link the biology of abscess formation with class II MHC involvement in "presenting" ZPS to T cells, Cobb et al. (2004) show that mice lacking inducible NO synthase (iNOS) failed to form abscesses when injected with intact ZPS, a situation reversed by administration of chemically cleaved ZPS. Similarly, processed radiolabeled ZPS could be isolated in association with class II MHC from wild-type splenocytes but not from iNOS deficient splenocytes. Here again, preprocessed ZPS restored class II MHC association. Thus, ZPS processing could occur as part of the inflammatory response.

It is almost 20 years since MHC molecules were shown to bind peptides with specificity that correlated with the variation in immune responses to protein antigens seen in MHC-different individuals (Babbitt et al., 1985; Buus et al., 1986). In the intervening years, the structure of MHC molecules and the details of their assembly with peptide have become part of the immunological landscape. Inevitably, the study reported today presents a less complete picture and it will be fascinating to see how the missing pieces in the story are filled in. In particular, it is unclear how processed ZPS interacts with class II MHC molecules and what the precise determinants of T cell activation are. Currently, it is known only that the zwitterionic character of ZPS is important and that T cell activation by ZPS falls off below the 10–15 kDa size range. Does MHC polymorphism influence ZPS binding? Are ZPS-associated MHC molecules also peptide occupied or does ZPS substitute for peptide in stabilizing the structure? Cobb et al. (2004) show that binding of processed ZPS to class II MHC in vitro requires the DM protein that assists conventional peptide loading. DM

may simply be exercising its well-documented capacity to prevent aggregation of peptide-free class II molecules. Whether DM is needed for T cell modulation of abscess formation in vivo is currently being established.

The helical structures for the two ZPSs reported to date seem too large to fit into the conventional MHC peptide binding groove. In their own previous reports, the authors suggest that the channels created by the helical twist of ZPSs might engage protein α helices (such as those that make up the groove) in an oblique fashion (Wang et al., 2000). Although it is not clear how specificity for MHC as opposed to other protein helices would be achieved in this binding mode, it is intriguing that the $\sim$20 Å pitch of the helical ZPS structure is about the same as the distance between the two MHC α helices. This or a related mode of binding might more readily explain why 10–15 kDa ($\sim$50 monosaccharide units) is required for optimal T cell stimulation i.e., considerably larger than a typical class II-associated peptide.

Aspects of ZPS ability to stimulate T cells are reminiscent of the bacterial superantigens that "crosslink" MHC and T cell receptor through interactions outside the MHC/peptide/TCR interface (Li et al., 1999). The authors resist a superantigen comparison based on the requirement for endosomal trafficking and processing of ZPSs, which superantigens do not share. Nonetheless, there are hints in the current data and elsewhere that the stochiometry of ZPS binding to class II MHC may be quite high and that ZPSs may engage a broad range of MHC/TCR combinations. For example, another recent report from this group indicates that many different TCR β chains can recognize ZPS/class II MHC structures (Stingele et al., 2004). Perhaps ZPSs are the first example of superantigen-like T cell stimulators that require processing.

It was already known that specific T cell receptors can accommodate a polysaccharide ligand; the glycopart of MHC groove bound glycopeptides can engage the T cell receptor and indeed define the specificity of the T cell (Rudd et al., 2001). What is new in Cobb et al.'s (2004) report is the protein-free and processing dependent mode of association of a polysaccharide with MHC molecules to form a T cell stimulatory structure. This new work extends the range of pathogen-derived material that can coopt the basic MHC fold to trigger T cell responses. In addition to peptides and glycopeptides, lipid and glycolipid antigens have emerged recently as very important triggers of T cell responses, for example against *Mycobacterial* species (Brigl and Brenner, 2004). These bind to members of the CD1 family of proteins that are class I MHC look-alikes but engage bacterial lipid products within the endosome/lysosome pathway, i.e., like class II MHC molecules. Further installments of the ZPS/class II MHC story, and the improved understanding of the pathogenic processes that organisms like *B. fragilis* elicit, will be awaited with interest.

Colin Watts
Division of Cell Biology and Immunology
Wellcome Trust Biocentre
University of Dundee
Dundee DD1 5EH
United Kingdom

Selected Reading

Babbitt, B.P., Allen, P.M., Matsueda, G., Haber, E., and Unanue, E.R. (1985). Nature *317*, 359–361.

Brigl, M., and Brenner, M.B. (2004). Annu. Rev. Immunol. *22*, 817–890.

Buus, S., Colon, S., Smith, C., Freed, J.H., Miles, C., and Grey, H.M. (1986). Proc. Natl. Acad. Sci. USA *83*, 3968–3971.

Choi, Y.H., Roehrl, M.H., Kasper, D.L., and Wang, J.Y. (2002). Biochemistry *41*, 15144–15151.

Cobb, B.A., Wang, Q., Tzianabos, A.O., and Kasperet, D.L. (2004). Cell, this issue, 677–687.

Kalka-Moll, W.M., Tzianabos, A.O., Bryant, P.W., Niemeyer, M., Ploegh, H.L., and Kasper, D.L. (2002). J. Immunol. *169*, 6149–6153.

Li, H., Llera, A., Malchiodi, E.L., and Mariuzza, R.A. (1999). Ann. Rev. Immunol. *17*, 435–466.

Rudd, P.M., Elliott, T., Cresswell, P., Wilson, I.A., and Dwek, R.A. (2001). Science *291*, 2370–2376.

Stingele, F., Corthesy, B., Kusy, N., Porcelli, S.A., Kasper, D.L., and Tzianabos, A.O. (2004). J. Immunol. *172*, 1483–1490.

Tzianabos, A.O., and Kasper, D.L. (2002). Curr. Opin. Microbiol. *5*, 92–96.

Wang, Y., Kalka-Moll, W.M., Roehrl, M.H., and Kasper, D.L. (2000). Proc. Natl. Acad. Sci. USA *97*, 13478–13483.