PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Molecular Chaperones and the Immune Response [and Discussion]

Douglas Young, Eulogia Roman, Carlos Moreno, Rebecca O'Brien, Willi Born, W. J. Welch, M.-J. Gething, P. Lund, A. R. Coates, I. G. Haas and P. Viitanen

Phil. Trans. R. Soc. Lond. B 1993 **339**, 363-368
doi: 10.1098/rstb.1993.0035

Molecular chaperones and the immune response

DOUGLAS YOUNG[1], EULOGIA ROMAN[1], CARLOS MORENO[1], REBECCA O'BRIEN[2] AND WILLI BORN[2]

[1]*MRC Tuberculosis and Related Infections Unit, Royal Postgraduate Medical School, Hammersmith Hospital, Ducane Road, London W12 0HS, U.K.*
[2]*National Jewish Center for Immunology and Respiratory Medicine, 1400 Jackson Street, Denver, Colorado 80206, U.S.A.*

SUMMARY

Molecular chaperones belonging to heat shock protein families have been identified as prominent antigens in the immune response to a wide variety of infections. Recognition of such highly conserved antigens may contribute to protective immunity but, in some circumstances, may also have pathological autoimmune consequences. Recognition of chaperones may be an inherent feature of the immune system. Peptide mapping experiments revealed an overlap between hsp 70-binding sites and immunodominant regions of three protein antigens, consistent with a possible functional activity for molecular chaperones in the processing and presentation of peptides during class II-restricted T lymphocyte responses. A functional role for molecular chaperones in antigen processing may be a factor which contributes to their immunogenicity.

1. MOLECULAR CHAPERONES AS ANTIGENS

(*a*) *An immune preference for molecular chaperones*

In mounting a response to infection, the immune system selects a range of antigenic components for recognition by antibodies and T lymphocytes. It is intriguing that, from the vast array of potential antigens expressed by an invading microorganism, molecular chaperones belonging to highly conserved heat shock protein families have been identified as prominent immune targets in a wide range of bacterial, protozoan and helminth infections (Young *et al.* 1990). In the case of pathogenic mycobacteria (the leprosy and tubercle bacilli), for example, it has been reported that as many as one in five of the mycobacteria-reactive T cells in immunized mice may be directed towards the bacterial 60 kDa heat shock protein (hsp 60 or chaperonin) (Kaufmann *et al.* 1987), while in the peripheral blood of a leprosy patient, one in three of the T cells responsive to *Mycobacterium leprae* recognized the 10 kDa chaperonin (Mehra *et al.* 1992). The mycobacterial 70 kDa heat shock protein (hsp 70) is also a prominent antigen, and has been used in diagnostic assays for detection of antibody responses in patients with leprosy and tuberculosis (Elsaghier *et al.* 1991; McKenzie *et al.* 1991).

What are the features of molecular chaperones which make them such appealing antigens? One might have anticipated that the immune system would respond most vigorously to the most exotic, species-specific proteins expressed by the invading pathogen, with the goal of efficiently distinguishing foreign organisms from self tissue and avoiding autoimmunity. The prominence of conserved molecular chaperones in the immune repertoire was therefore an unexpected finding which has triggered considerable interest amongst immunologists. The fact that some chaperones make up a major proportion (1–2%) of the total cell protein content, with the potential for further induction under stress conditions, could make an important quantitative contribution to their immunogenicity. Moreover it has been suggested that there may be an additional inherent bias introduced during development of the immune repertoire which favours recognition of conserved, 'self-like', proteins (Cohen & Young 1991). Support for this suggestion has been provided by a recent study in which the naive T cell repertoire in human cord blood was compared to the mature repertoire in the peripheral blood after exposure to microorganisms (Fischer *et al.* 1992). A high frequency of responses to the mycobacterial hsp 60 was found in the naive, unexposed, lymphocyte population, whereas responses to other mycobacterial antigens were elevated only in the mature peripheral repertoire after exposure to antigen. Similarly, the murine hsp 60-reactive γδ T cells discussed below are found in the new born thymus at a frequency comparable to that in peripheral adult organs (O'Brien *et al.* 1992). Recognition of molecular chaperones may be a feature which is somehow 'hard-wired' into the immune system at an early stage of development.

(*b*) *Sequence conservation, stress and autoreactivity*

In mounting a vigorous response to the highly

Phil. Trans. R. Soc. Lond. B (1993) **339,** 363–368
Printed in Great Britain

conserved chaperone proteins the immune system increases the risk that it may cross-react with the self homologue in addition to the closely related foreign antigen. Increased synthesis of hsp 70 and hsp 60 chaperones following exposure of cells to a wide variety of physiological stress stimuli (Morimoto *et al.* 1990) adds an additional dimension to their potential role as autoantigens, and the possible triggering of autoreactive responses directed to chaperone proteins overexpressed in infected cells has been widely discussed (Lamb *et al.* 1989; Koga *et al.* 1989). Human T cells which recognize antigenic determinants shared by microbial and self chaperones have been reported from many laboratories (see, for example, Lamb *et al.* (1989); Munk *et al.* (1989); Haregewoin *et al.* (1990)), and hsp 60-autoreactive T cells have been shown to modulate autoimmune phenomena in several animal models of disease (Cohen 1991). Although the level of such cells may be elevated in certain human autoimmune diseases (see De Graeff-Meeder *et al.* 1991), autoreactive cells are also found in healthy individuals and autoimmune disease is clearly not an inevitable consequence of autoreactivity. Autoreactivity may in fact be a purposeful and beneficial aspect of immunity, with a role in detection and elimination of infected or abnormal cells. Elevated immune expression of stress proteins could provide a signal to trigger recognition and elimination of such cells. Hsp 60-reactive γδ T cells may function in this type of 'immune surveillance' pathway (Born *et al.* 1990*b*).

(*c*) *Hsp 60-reactive γδ T cells*

Most T cells recognize antigen via a specific receptor molecule comprising two subunits, the α and the β chains. A second T cell subset, accounting for 1–5% of the total T cell population in rodents and humans, utilizes an alternative receptor made up of γ and δ chains (Brenner *et al.* 1988). In vivo, γδ T cells accumulate at sites of bacterial infection (Modlin *et al.* 1989) and are thought to play a protective role in the early activation of the immune response to infection (Hiromatsu *et al.* 1992). A major fraction of murine γδ T cells have receptors capable of recognizing the mycobacterial hsp 60 chaperonin (O'Brien *et al.* 1989, 1992), and the specificity of this response has been examined in detail. Do hsp 60-reactive γδ T cells recognize the microbial hsp 60 in infected lesions, or are they able to recognize self hsp 60 induced as a consequence of the infection?

Characteristically, hsp 60-reactive T cell hybridomas are spontaneously activated in the absence of added antigen, suggesting autorecognition of a self component, but can then be further stimulated by addition of peptide fragments from mycobacterial hsp 60 (Born *et al.* 1990*a*). The response is focused on one specific region of the protein – residues 180–190 in the mycobacterial sequence, with peptides of seven amino acid residues being identified as the minimum determinant (Born *et al.* 1993). Although there are a number of differences between the mammalian and bacterial sequences within this region, a peptide corresponding to the self sequence was able to stimulate the hsp 60-reactive hybridomas at a concentration comparable to that seen with the mycobacterial peptide (figure 1). Further studies will be required to determine whether the natural immunogen recognized *in vivo* is in fact the microbial or mammalian peptide, but these findings lend support to the concept that autoreactivity may sometimes play a beneficial role in protective immunity.

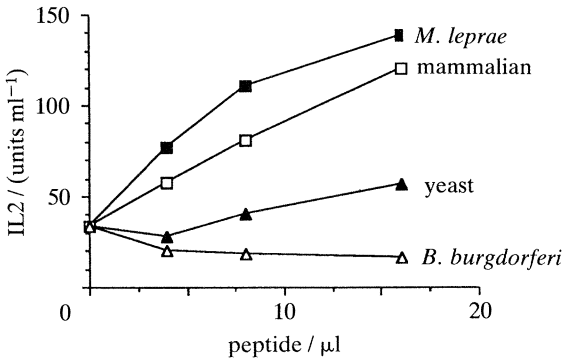

Figure 1. Recognition of hsp 60 peptides by γδ T cells. Interleukin 2 (IL2) secretion by an hsp 60-reactive murine γδ T cell hybridoma (74BAS86) was monitored after addition of peptides corresponding to the region 180–190 of the 65 kDa antigen (hsp 60) of *Mycobacterium leprae*. In each case, the addition of 1 μl of peptide solution resulted in a final concentration of 4 μl ml^{-1} in the assay. Peptides based on the mycobacterial and mammalian sequences induced similar responses despite a number of amino acid differences in this region (*M. leprae* sequence: TFGLQLELTEG: mammalian sequence: TLNDELEIIEG). In contrast, a peptide from the corresponding region of *Borrelia burgdorferi* hsp 60 failed to stimulate this hybridoma.

2. MOLECULAR CHAPERONES AND ANTIGEN PROCESSING

In evaluating the significance of immune responses to molecular chaperones, it is important to determine whether the precise epitope recognized is in a variable or conserved region of the protein. How does the immune system select particular regions of an antigen for T cell recognition? Processing and presentation of antigens for T cell recognition involves polypeptide unfolding, partial degradation and transfer between intracellular compartments, all features which suggest a possible functional role for molecular chaperones.

T lymphocytes recognize peptide fragments bound to cell surface proteins belonging to the major histocompatibility complex (MHC). Within the MHC locus, two classes of proteins which present antigens to T cells have been characterized. Class I proteins are found on all cells as a heterodimer consisting of an α chain paired with β2-microglobulin. The crystal structure of MHC class I proteins shows the presence of a specific cleft in which the antigenic peptide is bound (Bjorkman *et al.* 1987). Class II MHC proteins are generally found on specialized antigen-presenting cells and comprise a heterodimer of α and β subunits. Structural modelling suggests that class II molecules have peptide binding sites similar to those of class I

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

(Brown *et al.* 1988). Sequence similarity and structural modelling also reveal a relationship between the peptide binding cleft of MHC molecules and the C terminal portion of molecular chaperones belonging to the hsp 70 family (Flajnik *et al.* 1991). Hence, the machinery for peptide presentation to immune cells may have evolved by exploitation and refinement of the peptide binding properties of molecular chaperones.

The ability of a peptide to bind to a particular MHC molecule is obviously essential for immunogenicity but additional factors also influence the pattern of epitope recognition. Separate pathways are involved in delivery of peptides to the different classes of MHC molecules. Antigens synthesized within the cell cytoplasm, viral antigens for example, are generally presented by class I molecules; antigens taken up from the extracellular medium by endocytosis tend to associate with class II molecules (Germain 1986). Proteins destined for class I presentation are partially degraded by proteasome complexes containing subunits encoded by genes located within the MHC region of the chromosome (reviewed by Goldberg & Rock (1992)); peptide fragments are then transported into the endoplasmic reticulum by transporter proteins, which are again encoded within the MHC region (Deverson *et al.* 1990; Trowsdale *et al.* 1990). The final antigen–MHC class I complex contains peptides of eight or nine amino acids (Rotzschke *et al.* 1990; Jardetsky *et al.* 1991). Targeting of proteins for proteasome digestion is generally mediated by addition of ubiquitin to their amino termini, a mechanism which may also apply to antigen processing (Townsend *et al.* 1988), but it is not clear whether there is a mechanism by which proteins are separated into those destined for degradation to amino acids and those which are preserved as antigenic peptides. Similarly, the mechanism which allows foreign antigens to compete for presentation with the overwhelming bulk of self proteins remains to be identified.

The processing pathway for peptides associating with MHC class II is less well understood (reviewed by Unanue (1992)). Lysosomal degradation has been implicated in generation of peptides for class II binding, and the substrate specificity of acid proteases may play a role in shaping the class II repertoire (Vidard *et al.* 1992). It is not known how degradation is arrested at the peptide level for those fragments destined for immune presentation. It appears that peptides bound to MHC class II proteins are longer and more heterogeneous than those bound by MHC class I proteins, generally in the region of 13 to 17 amino acids (Rudensky *et al.* 1991). Several lines of evidence suggest a possible role for hsp 70 in the class II pathway (De Nagel & Pierce 1992). Antibodies to hsp 70 were found to block presentation of an antigenic peptide to a class II-restricted T cell hybridoma, for example (Lakey *et al.* 1987), and hsp 70 has been implicated in delivery of proteins for lysosomal degradation (Chiang *et al.* 1989). Immunoelectron microscopy has been used to demonstrate the presence of hsp 70 within the endocytic vesicles thought to be the site of assembly of peptide–class II complexes (Van Buskirk *et al.* 1991). Finally, as in the case of the class I processing-related proteins, at least two hsp 70 genes are located in the MHC region of the chromosome (Sargent *et al.* 1989). To further explore the possible relationship between hsp 70 and antigen processing, we have characterized hsp 70 binding sites on a series of protein antigens for which the T cell epitope profile has already been established.

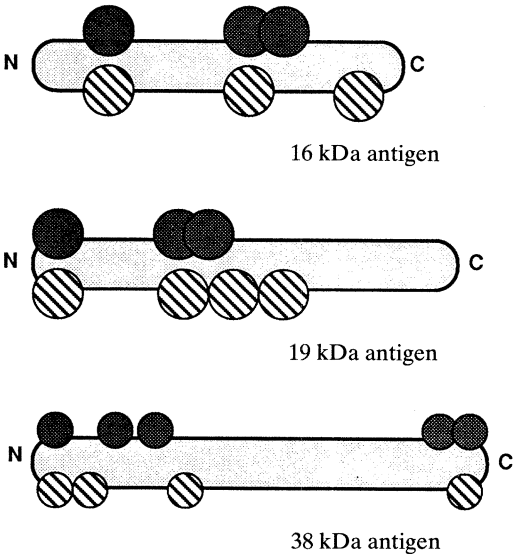

Figure 2. Mapping of hsp 70 binding sites on protein antigens. Hsp 70 binding sites on three protein antigens were mapped using synthetic peptides and compared with sites involved in class II-restricted T cell recognition of the same antigens. Hsp 70 sites are indicated by hatched circles, with filled circles indicating immunodominant regions of the proteins inducing proliferative responses in the context of multiple human and murine MHC class II haplotypes.

(*a*) *Mapping hsp 70 binding sites on protein antigens*

Using a set of randomly synthesized peptides, Flynn *et al.* (1991) demonstrated that BiP – the hsp 70 protein from the endoplasmic reticulum – preferentially recognizes stretches of seven amino acids rich in aliphatic amino acids. We have investigated whether a similar specificity holds for other members of the hsp 70 family, and whether there is any topological relationship between hsp 70 binding sites and regions of protein antigens involved in T cell recognition.

Sets of overlapping 20-mer peptides corresponding to the sequences of three well-characterized protein antigens were tested for their ability to compete with a biotinylated control peptide for binding to BiP and to a prokaryotic hsp 70 protein from *Mycobacterium tuberculosis* (Roman *et al.* 1993). For each of the three antigens, preferential hsp 70 binding sites were identified. In most instances binding to bacterial hsp 70 coincided with binding to BiP, indicating an overall similarity in peptide specificity for different hsp 70 family members. Some variations between the two proteins were detected, suggestive of limited differences in fine specificity within the hsp 70 family. The

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

same sets of peptides have previously been tested for their recognition by class II-restricted T cells, and immunodominant regions recognized in the context of a range of human and murine MHC haplotypes have been identified (Faith *et al*. 1991; Harris *et al*. 1991, 1993; Vordermeier *et al*, 1991, 1993). Comparison of T cell epitopes with hsp 70 binding sites revealed overlap in some instances (figure 2), consistent with a possible role for hsp 70 binding in preferential protection or transport of peptides destined for class II presentation. Although the presenting molecule involved in γδ T cell recognition has not yet been identified, it is intriguing to note that the minimal 7-mer epitope from the mycobacterial hsp 60 antigen recognized by γδ T cells binds strongly to hsp 70 (Born *et al*. 1993).

3. CONCLUDING REMARKS

Molecular chaperones may play both an active and a passive role in the immune response: by their chaperone function in antigen processing pathways and by their ability to act as targets for immune recognition. It is attractive to suggest that these two aspects may be linked, with an antigen processing role providing preferential access to MHC molecules and perhaps contributing to the development of a bias in the immune repertoire in favour of members of the conserved chaperone families. Identification of fragments of hsp 70 (Nelson *et al*. 1992) and hsp 90 (Jardetsky *et al*. 1991) among the self peptides which occupy the binding site of MHC molecules in uninfected cells is consistent with this hypothesis. It should be borne in mind, however, that, while a role in antigen processing is most readily envisaged for members of the hsp 70 family, it is hsp 60 which has been the focus of most studies of chaperone immunogenicity.

REFERENCES

Bjorkman, P.J., Saper, M.A., Samraoui, B., Bennett, W.S., Strominger, J.L. & Wiley, D.C. 1987 Structure of the human class I histocompatibility antigen, HLA-A2. *Nature*, *Lond*. **329**, 506–512.

Born, W., Hall, L., Dallas, A., *et al*. 1990*a* Recognition of a peptide antigen by heat shock-reactive γδ T lymphocytes. *Science*, *Wash*. **249**, 67–69.

Born, W., Happ, M.P., Dallas, A., *et al*. 1990*b* Recognition of heat shock proteins and γδ cell function. *Immunol*. *Today* **11**, 40–43.

Born, W., Vollmer, M., Fu, Y.-X., *et al*. 1993 Response of γδ T cell hybridomas to peptides of seven amino acids. (Submitted.)

Brenner, M.B., Strominger, J.L. & Krangel, M.S. 1988 The γδ T cell receptor. *Adv*. *Immunol*. **43**, 133–192.

Brown, J.H., Jardetsky, T., Saper, M.A., Samraoui, B., Bjorkman, P.J. & Wiley, D.C. 1988 A hypothetical model of the foreign antigen binding site of class II histocompatibility molecules. *Nature*, *Lond*. **332**, 845–850.

Chiang, H.L., Terlecky, S.R., Plant, C.P. & Dice, J.F. 1989 A role for a 70-kilodalton heat shock protein in lysosomal degradation of intracellular proteins. *Science*, *Wash*. **24**, 382–385.

Cohen, I.R. 1991 Autoimmunity to chaperonins in the pathogenesis of arthritis and diabetes. *A*. *Rev*. *Immunol*. **9**, 567–589.

Cohen, I.R. & Young, D.B. 1991 Autoimmunity, microbial immunity and the immunological homuniculus. *Immunol*. *Today* **12**, 105–110.

De Graeff–Meeder, E.R., Van der Zee, R., Rijkes, G.T. *et al*. 1991 Recognition of human 60 kD heat shock protein by mononuclear cells from patients with juvenile chronic arthritis. *Lancet* **337**, 1368–1372.

De Nagel, D.C. & Pierce, S.K. 1992 A case for chaperones in antigen processing. *Immunol*. *Today*. **13**, 86–89.

Deverson, E.V., Gow, I.R., Coadwell, W.J., Monaco, J.J., Butcher, G.W. & Howard, J.C. 1990 MHC class II region genes encoding proteins related to the multidrug resistance family of transmembrane transporters. *Nature*, *Lond*. **348**, 738–741.

Elsaghier, A.A.F., Wilkins, E.G.L., Mehrotra, P.K., Jindal, S. & Ivanyi, J. 1991 Elevated antibody levels to stress protein hsp 70 in smear-negative tuberculosis. *Immunol*. *infect*. *Dis*. **1**, 323–328.

Faith, A., Moreno, C., Lathigra, R. *et al*. 1991 Analysis of human T-cell epitopes in the 19,000 MW antigen of *Mycobacterium tuberculosis*: influence of HLA-DR. *Immunology* **74**, 1–7.

Fischer, H.P., Sharrock, C.E.M. & Panayi, G.S. 1992 High frequency of cord blood lymphocytes against mycobacterial 65-kDa heat-shock protein. *J*. *Immunol*. **22**, 1667–1669.

Flajnik, M.F., Canel, C., Kramer, J. & Kasahara, M. 1991 Which came first, MHC class I or class II? *Immunogenetics* **33**, 295–300.

Flynn, G.C., Pohl, J., Flocco, M.T. & Rothman, J.E. 1991 Peptide binding specificity of the molecular chaperone BiP. *Nature*, *Lond*. **353**, 726–730.

Germain, R.N. 1986 The ins and outs of antigen processing and presentation. *Nature*, *Lond*. **322**, 687–689.

Goldberg, A.L. & Rock, K.L. 1992 Proteolysis, proteasomes and antigen presentation. *Nature*, *Lond*. **357**, 375–379.

Haregewoin, A., Singh, B., Gupta, R.S. & Finberg, R.W. 1990 A mycobacterial heat shock protein responsive γδ T cell clone also responds to the homologous human heat shock protein: a possible link between infection and autoimmunity. *J*. *infect*. *Dis*. **163**, 156–159.

Harris, D.P., Vordermeier, H.M., Friscia, G. *et al*. 1993 Adjacent topography of genetically permissive, immunodominant epitopes of the 19 kDa antigen of *Mycobacterium tuberculosis* recognised by human and murine T cells. (Submitted.)

Harris, D.P., Vordermeier, H.M., Roman, E. *et al*. 1991 Murine T cell stimulatory peptides from the 19-kDa antigen of *Mycobacterium tuberculosis*. *J*. *Immunol*. **147**, 2706–2712.

Hiromatsu, K., Yoshikai, Y., Matsuzaki, G. *et al*. 1992 A protective role for γδ T cells in primary infection with *Listeria monocytogenes* in mice. *J*. *exp*. *Med*. **175**, 49–56.

Jardetsky, T.S., Lane, W.S., Robinson, R.A., Madden, D.R. & Wiley, D.C. 1991 Identification of self peptides bound to purified HLA-B27. *Nature*, *Lond*. **353**, 326–329.

Kaufmann, S.H.E., Vath, U., Thole, J.E.R., Van Embden, J.D.A. & Emmrich, F. 1987 Enumeration of T cells reactive with *Mycobacterium tuberculosis* organisms and specific for the 64-kDa protein. *Eur*. *J*. *Immunol*. **17**, 351–357.

Koga, T., Wand-Wurttenberger, A., de Bruyn, J., Munk, M.E., Schoel, B. & Kaufmann, S.H.E. 1989 T cells against a bacterial heat shock protein recognize stressed macrophages. *Science*, *Wash*. **246**, 1112–1115.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Lakey, E.K., Margoliash, E. & Pierce, S.K. 1987 Identification of a peptide binding protein that plays a role in antigen presentation. *Proc. natn. Acad. Sci. U.S.A.* **84**, 1659–1663.

Lamb, J.R., Bal, V., Mendez–Samperio, P. *et al.* 1989 Stress proteins may provide a link between the immune response to infection and autoimmunity. *Int. Immunol.* **1**, 191–196.

McKenzie, K.R., Adams, E., Britton, W.J., Garsia, R.J. & Basten, A. 1991 Sequence and immunogenicity of the 70-kDa heat shock protein of *Mycobacterium leprae*. *J. Immunol.* **60**, 1170–1177.

Mehra, V., Bloom, B.R., Bajardi, A.C. *et al.* 1992 A major T cell antigen of *Mycobacterium leprae* is a 10-kD heat-shock cognate protein. *J. exp. Med.* **175**, 275–284.

Modlin, R.L., Pirmez, C., Hofman, F.M. *et al.* 1989 Lymphocytes bearing antigen-specific γδ T-cell receptors accumulate in human infectious disease lesions. *Nature, Lond.* **339**, 544–548.

Morimoto, R.I., Tissieres, A. & Georgopoulos, C. (eds) 1990 *Stress proteins in biology and medicine*. New York: Cold Spring Harbor Laboratory Press.

Munk, M.E., Schoel, B., Modrow, S., Karr, R.W., Young, R.A. & Kaufmann, S.H.E. 1989 Cytolytic T lymphocytes from healthy individuals with specificity for self epitopes shared by the mycobacterial and human 65 kDa heat shock protein. *J. Immunol.* **143**, 2844–2849.

Nelson, C.A., Roof, R.W., McCourt, D.W. & Unanue, E.R. 1992 Identification of the naturally processed form of hen egg white lysozyme bound to the murine major histocompatibility complex class II molecule I-A^k. *Proc. natn. Acad. Sci. U.S.A.* **89**, 7380–7383.

O'Brien, R.L., Happ, M.L., Dallas, A., Palmer, E., Kubo, R. & Born, W.K. 1989 Stimulation of a major subset of lymphocytes expressing T cell receptor γδ by an antigen derived from *Mycobacterium tuberculosis*. *Cell* **57**, 667–674.

O'Brien, R.L., Fu, Y.-X., Cranfill, R. *et al.* 1992 Hsp 60-reactive γδ cells: a large, diversified T lymphocyte subset with highly focused specificity. *Proc. natn. Acad. Sci. U.S.A.* **89**, 4348–4352.

Roman, E., Moreno, C. & Young, D. 1993 Mapping of hsp 70 binding sites on protein antigens. (Submitted.)

Rotzschke, O., Falk, K., Deres, K. *et al.* 1990 Isolation and analysis of naturally processed viral peptides recognized by cytotoxic T cells. *Nature, Lond.* **348**, 253–255.

Rudensky, A.Y., Preston-Hurlburt, P., Hong, S.-C., Barlow, A. & Janeway, C.A. 1991 Sequence analysis of peptides bound to MHC class II molecules. *Nature, Lond.* **353**, 622–627.

Sargent, C.A., Dunham, I., Trowsdale, J. & Campbell, R.D. 1989 Human major histocompatibility complex contains genes for the major heat shock protein hsp 70. *Proc. natn. Acad. Sci. U.S.A.* **86**, 1986–1972.

Townsend, A., Bastin, J., Gould, K. *et al.* 1988 Defective presentation to class I-restricted cytotoxic T lymphocytes in vaccinia-infected cells is overcome by enhanced degradation of antigen. *J. exp. Med.* **168**, 1211–1224.

Trowsdale, J., Hanson, I., Mockridge, I., Beck, S., Townsend, A. & Kelly, A. 1990 Sequences encoded in the class II region of the MHC related to the 'ABC' superfamily of transporters. *Nature, Lond.* **348**, 741–744.

Unanue, E.R. 1992 Cellular studies on antigen presentation by class II MHC molecules. *Curr. Opin. Immunol.* **4**, 63–69.

Van Buskirk, A.M., De Nagel, D.C., Guagliardi, L.E., Brodsky, F.M. & Pierce, S.K. 1991 Cellular and subcellular distribution of PBP72/74, a peptide-binding protein that plays a role in antigen processing. *J. Immunol.* **146**, 500–506.

Vidard, L., Rock, K.L. & Benacerraf, B. 1992 Diversity in MHC class II ovalbumin T cell epitopes generated by distinct proteases. *J. Immunol.* **149**, 498–504.

Vordermeier, H.M., Harris, D.P., Lathigra, R., Roman, E., Moreno, C. & Ivanyi, J. 1993 Focusing of the murine T cell immune response after tuberculosis infection. Analysis of epitopes of the 16 kDa antigen. (Submitted.)

Vordermeier, H.M., Harris, D.P., Roman, E., Lathigra, R., Moreno, C. & Ivanyi, J. 1991 Identification of T cell stimulatory peptides from the 38-kDa protein of *Mycobacterium tuberculosis*. *J. Immunol.* **147**, 1023–1029.

Young, D.B., Mehlert, A. & Smith, D.F. 1990 Stress proteins and infectious diseases. In *Stress proteins in biology and medicine* (ed. R. I. Morimoto, A. Tissieres & C. Georgopoulos), pp. 131–165. New York: Cold Spring Harbor Laboratory Press.

Discussion

W. J. Welch (*Department of Medicine and Physiology, University of California, San Francisco, U.S.A.*). Has it been shown in the case of MHC class I that the α and β subunits are retained in the endoplasmic reticulum lumen because of binding to a chaperone?

D. B. Young. There is no definitive evidence on this point to my knowledge.

W. J. Welch. Is it known whether in the case of MHC class II the peptides that are presented are those that BiP may either bind or not bind?

M.-J. Gething (*Howard Hughes Medical Institute, University of Texas, Dallas, U.S.A.*). I have examined the published sequences for class II and sometimes they fit our motif for binding to BiP, but not always.

W. J. Welch. Maybe it is those that do not bind to BiP that bind to class I.

P. Lund (*School of Biological Sciences, University of Birmingham, U.K.*). Is there any direct evidence that components of the mitochondria are presented to the immune system on the surface of stressed cells, by perhaps using monoclonal antibodies against different mitochondrial proteins?

D. B. Young. There is indirect evidence that hsp 60-reactive cytotoxic T cells recognize stress macrophages. The immunologically relevant peptides are those in the cleft of the MHC molecule and it is difficult to design ways of detecting these.

A. R. Coates (*St George's Hospital Medical School, London, U.K.*). It has been shown that the chaperonins and other chaperones are strong stimulators of the immune system. Do the authors see any practical application of this observation?

D. B. Young. It is difficult to show protection against infection by using the chaperonins and the reason for this is that the native immunity involving these proteins is already high. One can see protection in the negative experiments, e.g. protection against infection is lost when γδ T cells are deleted. The challenge is to

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

see whether we can boost the general level of immunity against a range of bacterial infections. This is an attractive possibility from the vaccination standpoint but there is no evidence at the present that this approach will be successful.

I. G. Haas (*Institute of Genetics, University of Cologne, F.R.G.*). Is it known whether tumour cells present peptides from the hsp 70 family to the immune system? If γδ T cells protect from self-damage one might expect them to kill tumour cells in a self-restricted manner.

D. B. Young. I suspect that the γδ T cells recognize tumour cells and not normal cells because the tumour cells present the self-antigen more efficiently than normal cells.

P. Viitanen (*Du Pont de Nemours, Wilmington, U.S.A.*). In the peptide binding studies how do the authors correct for possible differences in the accessibility of some structures, especially when they occur as part of larger sequences?

D. B. Young. It may be that there is no primary structure requirement for binding but that the most extended structure is the one that most readily occupies the groove. Presumably the groove is a certain size and when other residues are present on either side of the groove there may be some steric or charge interaction that affects the binding. I accept your point that the structure of the peptide may be as important as its sequence.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES