

- (1991) *Science* 254, 1643–1647
- 11 Rosenberg, S.A., Spiess, P.J. and Lafreniere, R. (1986) *Science* 233, 1318–1321
- 12 Mueller, D.L., Jenkins, M.K. and Schwartz, R.H. (1989) *Annu. Rev. Immunol.* 7, 445–480
- 13 Liu, Y. and Linsley, P.S. (1992) *Curr. Opin. Immunol.* 4, 265–270
- 14 Linsley, P.S. and Ledbetter, J.A. (1993) *Annu. Rev. Immunol.* 11, 191–212
- 15 Chen, L., Mizuno, M.T., Singhal, M.C. *et al.* (1992) *J. Immunol.* 148, 2617–2621
- 16 Chen, L., Ashe, S., Brady, W.A. *et al.* (1992) *Cell* 71, 1093–1102
- 17 Townsend, S.E. and Allison, J.P. (1993) *Science* 259, 368–370
- 18 Baskar, S., Ostrand-Rosenberg, S., Nabavi, N. *et al.* (1993) *Proc. Natl Acad. Sci. USA* 90, 5687–5690
- 19 Schwartz, R.H. (1992) *Cell* 71, 1065–1068
- 20 Freeman, G.J., Greedman, A.S., Segil, J.M. *et al.* (1989) *J. Immunol.* 143, 2714–2722
- 21 Otten, G.R. and Germain, R.N. (1991) *Science* 251, 1228–1231
- 22 Harding, F.A., McArthur, J.G., Gross, J.A. *et al.* (1992) *Nature* 356, 607–609
- 23 Miller, J.F.A.P., Morahan, G., Allison, J. and Hoffmann, M. (1991) *Immunol. Rev.* 122, 103–116
- 24 Muller, H.K., Bucana, C. and Kripke, M.L. (1992) *Semin. Immunol.* 4, 205–215

S-type mammalian lectins in allergic inflammation

Fu-Tong Liu

Our understanding of the mechanism of allergic disease is continuously influenced by new developments in the bio-medical sciences. The studies of glycoconjugates and animal lectins have emerged as an exciting new frontier. One family of animal lectins, soluble lactose-binding lectins, has been studied extensively by a number of laboratories. Evidence is mounting that members of this family of lectins exist in the extracellular space and may be capable of affecting functions of various cells. In this article Fu-Tong Liu presents a revised view of allergic inflammation with emphasis on the modulatory effect of soluble lectins on mast-cell function.

Carbohydrate structures on various glycoconjugates are believed to be important in diverse biological processes including cell differentiation, cell–cell interaction and oncogenesis¹. The finding of a large number of animal lectins that recognize specific complementary glycoconjugates has provided a key to understanding the function of various carbohydrate structures². One family of lectin, designated as soluble lectins^{3,4}, characterized by its soluble nature and lactose-binding activity, is particularly noteworthy, as these lectins have been implicated in a variety of biological processes. This review emphasizes how mammalian lectins may influence allergic inflammation, using one of the soluble lectins, IgE-binding protein (εBP), as an example.

The 31 kDa εBP was first identified in rat basophilic leukemia cells^{5,6} and cloning and sequencing of the cDNA revealed a novel sequence with several interesting structural features^{6–8}. It is now clear that the protein is identical to an animal lectin CBP 35 and has an intrinsic galactose-binding activity^{9,10}. The lectin has been designated by various other names such as lung lectins (HL-29 or RL-29)^{11,12}, metastasis-associated lectin (L-34)¹³ and the Mac-2 antigen, a

murine macrophage cell-surface protein¹⁴. There has been an exponential growth in our knowledge of this animal lectin and the possible roles of this protein in regulation of cell growth¹⁵, RNA splicing¹⁶, cell adhesion¹⁷ and tumor metastasis¹⁸.

Biochemical properties of εBP

Protein sequences of εBP from various species have been elucidated, either from DNA cloning or direct protein sequencing.

εBP contains two domains

One characteristic feature of εBP is that it has a two-domain structure (Fig. 1(a)). The carboxy-terminal domain contains both hydrophobic and hydrophilic regions typical of globular proteins, while the amino-terminal domain is composed of tandem repeats of a highly conserved sequence of nine amino acids, Tyr-Pro-Gly-Pro(Gln)-Ala(Thr)-Pro(Ala)-Pro-Gly-Ala⁷. The cDNA coding for human εBP has recently been cloned^{19–22} and a comparison of the deduced sequence of human εBP with that of rat and mouse εBP revealed that the two-domain structure and protein sequence of this lectin are highly conserved.

εBP is a soluble lectin containing an S-type carbohydrate-recognition domain

Animal lectins have been classified according to the type of carbohydrate-recognition domains (CRD) they contain²³. One is C-type (calcium-dependent), a notable example being the cell adhesion proteins, selectins²⁴. The second is an S-type (soluble) with the classical example being the 14 kDa soluble galactose-binding lectins²⁵⁻²⁷. Recently, another 14 kDa lectin²⁸ and a 36 kDa²⁹ lectin have been added to this family. The carboxyl-terminal domain of *εBP* shares significant sequence similarity with the lectin domains of all these lectins^{8,29,30}.

Carbohydrate specificity of *εBP*

The carbohydrate specificity of RL-29 and HL-29 (identical to rat and human *εBP*) have been extensively studied^{11,12}. Although S-type lectins are generally referred to as galactoside-binding lectins, they exhibit selectivity for distinct oligosaccharide structures. For example, the relative affinity of various saccharides for RL-29 is Gal(1) < Galβ1-3GalNAc(7) < Galβ1-4Glc(100) < Galβ1-3GlcNAc(270) < Galβ1-4GlcNAc(700) < GalNAcα1-3(Fucα1→2)Galβ1-4Glc(2500). The numbers in parenthesis refer to the relative affinity of the saccharides for RL-29 (Ref. 12).

Properties of *εBP* that support its extracellular functions

Existence of *εBP* in the extracellular media

Neither the 31 kDa (*εBP*) nor the 14 kDa S-lectins contain a classical signal sequence. Nevertheless, the possible extracellular functions of this class of lectin have been discussed³. Barondes and co-workers³ have studied soluble animal lectins extensively and have provided evidence for extracellular functions of certain S-type lectins. Some of these lectins are initially concentrated inside the cells, but are eventually exported³. In particular, Cooper and Barondes³¹ demonstrated, by both immunoprecipitation of metabolically labeled protein and immunohistochemical localization, externalization of a 14 kDa S-type lectin from mouse muscle cells. Their data suggest that the lectin becomes concentrated in evaginations of plasma membrane, which pinch off to form lectin-rich extracellular vesicles.

The level of surface *εBP* on rat basophilic leukemia (RBL) cells increases when the cells are activated by IgE immune complexes³² and, from pulse-chase analysis, it has been shown that the Mac-2/*εBP* is secreted by activated macrophages¹⁴. *εBP* is detected in the peritoneal lavage fluid from thioglycollate-stimulated mice but not in untreated mice (L.G. Frigeri and F.T. Liu, unpublished). Finally, it is important to point out that the carbohydrate binding of *εBP* is not dependent on the presence of reducing agents³³, in contrast to the 14 kDa S-lectin^{34,35}. Thus, *εBP* can function in extracellular oxidative environments.

Multivalent property of *εBP*

Like many other lectins, *εBP* behaves at least bivalently, as best demonstrated by its ability to agglutinate erythrocytes³³. Both recombinant *εBP* and the

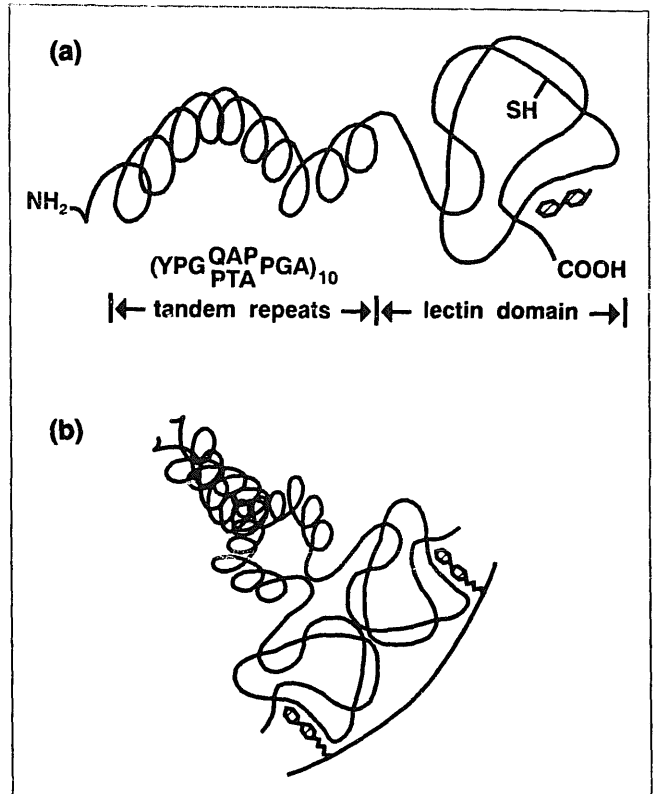


Fig. 1. (a) A model for *εBP* structure. A two-domain structure of *εBP* is depicted. The amino-terminal domain, which consists of tandem repeats of nine amino acids (Tyr-Pro-Gly-Gln/Pro-Ala/Thr-Pro/Ala-Pro-Gly-Ala), is presented as a hypothetical helical structure with many turns. The carboxy-terminal domain (the lectin domain) is viewed as a typical globular structure. A single cysteine residue in the carboxy-terminal domain is shown (SH). A pair of solid hexagons indicates the putative carbohydrate-binding site of *εBP*. (b) Model of *εBP* self-association resulting in a dimer with a much higher affinity than the monomer for a bivalent ligand. A schematic diagram illustrating noncovalent homodimerization of *εBP* involving the amino-terminal domain. When *εBP* encounters a bivalent ligand the molecule binds to the ligand much more tightly than would be expected for monomers because of the protein-protein self-association.

carboxy-terminal domain fragment (*εBP*-C) contain only one lactose-binding site³⁶. However, *εBP*-C differs from *εBP* in that the former does not cause hemagglutination.

An important clue for the basis of the multivalency was discovered when analysing *εBP* binding to solid-phase IgE. ¹²⁵I-labeled *εBP* was exposed to IgE coated on microtiter plates in the presence of various concentrations of *εBP* and *εBP*-C. As expected, *εBP*-C inhibited the binding effectively in a dose-dependent fashion. In contrast however, an increase in *εBP* concentrations enhanced the binding of ¹²⁵I-*εBP* to IgE, reflecting cooperative binding thus suggesting that *εBP* self-associates and that this property is dependent on the amino-terminal portion of *εBP* (Ref. 36). The tendency of *εBP* to self associate was substantiated by a chemical crosslinking study³⁶. Positive cooperativity in binding of L-29 (identical to *εBP*) to laminin was also reported and the requirement of the amino-terminal

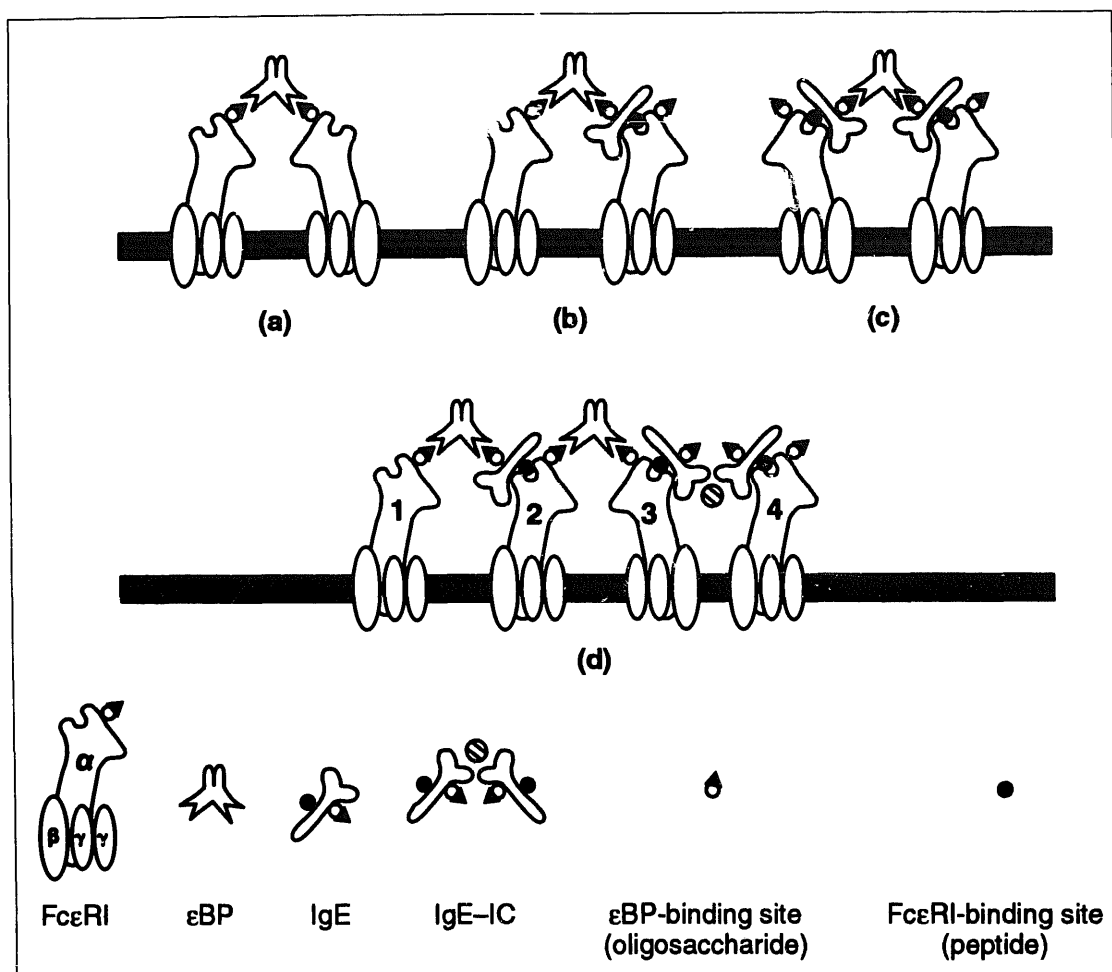


Fig. 2. Facilitation of IgE receptor aggregation by ϵ BP. This figure is a schematic drawing of the IgE receptor aggregation facilitated by ϵ BP. Diagrams (a), (b) and (c) represent three ways in which ϵ BP could crosslink with Fc ϵ RI. Diagram (d) represents an example of Fc ϵ RI aggregation facilitated by ϵ BP. The ϵ BP molecule brings receptors 1 and 2 into proximity of receptors 3 and 4 that are bridged by an IgE immune complex. For simplicity, the binding of ϵ BP to other glycoproteins is not included in the diagram. The various structures are labeled at the bottom of the figure.

domain for the observed positive cooperativity was also noted³⁷.

From these results, it seems that ϵ BP forms dimers or oligomers through noncovalent inter-molecular association involving the amino-terminal domain. The amino-terminal domain is composed of proline/glycine-rich tandem repeats and it is possible that this domain forms a unique tertiary structure that is predisposed to intermolecular association. Moreover, the self-association constant for ϵ BP must be low, since stable ϵ BP dimers or oligomers could not be detected in the absence of chemical crosslinkers. However, protein self association may be facilitated as ϵ BP binds to multivalent ligands, resulting in highly stable complexes (Fig. 1(b)).

Recognition of specific glycoproteins by ϵ BP

ϵ BP binds to the surface of various cell types *via* its lectin function³². Efforts to identify specific cell surface glycoproteins on RBL cells that are recognized by ϵ BP,

revealed that ϵ BP binds only a small number of glycoprotein species³⁸. Significantly, when other cell lines were analysed in the same manner, simple patterns were also seen (authors' unpublished observations). The finding of a limited number of proteins that bind to ϵ BP in the different cell types examined indicated a high selectivity of glycoprotein recognition by ϵ BP. Rosenberg *et al.*³⁹ reported that Mac-2 (identical to ϵ BP) binds two glycoproteins from human colon adenocarcinoma cell lines, a result that also supports the restricted glycoprotein recognition by this type of lectin.

The possible role of ϵ BP in allergic inflammation

The IgE-binding function of ϵ BP alone already points to the possible relevance of this protein in IgE-mediated allergic reactions. A recent development further supports an important role of ϵ BP in allergic inflammation: on mast cells, the IgE receptor (Fc ϵ RI)

is one of the major glycoproteins recognized by ϵ BP³⁸. On the basis of this finding, as well as the properties of ϵ BP described in the previous section, it should be evident that ϵ BP has a potential to modulate the functions of mast cells. Two possible models have been considered.

First, ϵ BP activates mast cells by bridging IgE receptors, receptor-bound IgE, or IgE and receptor. Because ϵ BP binds both IgE and Fc ϵ RI, and because it can form dimers or even oligomers, the following situations may be envisaged: (1) ϵ BP crosslinks two or more Fc ϵ RI; (2) ϵ BP crosslinks two or more receptor-bound IgE; and (3) ϵ BP bridges receptor-bound IgE and Fc ϵ RI (Fig. 2, (a), (b) and (c)). Since it is well known that receptor aggregation is a necessary and sufficient signal for mast-cell activation⁴⁰, all of these above situations could lead to mast-cell activation.

Second, ϵ BP modulates Fc ϵ RI-mediated mast-cell activation through binding to Fc ϵ RI or IgE. For example, by coupling two or more IgE receptor molecules, ϵ BP may augment receptor aggregation induced by IgE immune complexes (Fig. 2(d)).

It is important to point out that the capacity to bind both IgE and Fc ϵ RI is most likely a unique property of ϵ BP. Of the several soluble lactose-binding lectins, ϵ BP appears to be the only one that binds IgE (Ref. 41). Furthermore, preliminary data support the biological effects of ϵ BP on RBL cells. Thus, when RBL cells were first treated with ϵ BP and then sensitized with IgE (DNP-specific), a 30–160% increase in serotonin release induced by antigen (DNP-BSA) was observed (authors' unpublished results). In addition, in two different sets of experimental conditions, ϵ BP was noted to cause serotonin release from unsensitized or IgE-sensitized RBL cells (Ref. 38 and R.I. Zuberi, L.G. Frigeri and F-T. Liu, unpublished). Therefore, it appears that ϵ BP can affect the activation of mast cells and, on the basis of these observations, the following hypothesis has been formulated: inflammatory stimuli cause macrophages or mast cells to release ϵ BP, which in turn may bind to and activate other mast cells present at the inflammatory site. In this way, ϵ BP contributes to amplification of an inflammatory response. An important implication of this model is that it is possible that simple oligosaccharide ligands for ϵ BP may be capable of attenuating inflammation and thus have utility as novel therapeutic agents.

Broad-spectrum modulatory function of ϵ BP

The effect of ϵ BP will probably not be restricted to mast cells, as glycoprotein ligands for ϵ BP are probably present on many other cell types. Since ϵ BP functions at least bivalently, it has the potential to link two glycoproteins that share oligosaccharide chains recognized by ϵ BP on any cell type, and thus ϵ BP may modulate the functions associated with these glycoproteins. The effect is expected to be broad because ϵ BP has wide tissue distribution⁴² and each cell type may have a different set of ϵ BP-reactive glycoconjugates linked to a spectrum of cellular functions. In addition, ϵ BP may be involved in inflammatory responses by a mechanism other than that described in this article. Human neutrophils were found to express ϵ BP and a role for

ϵ BP in IgE-dependent activation of neutrophils has been indicated⁴³. The role of lectins in modulating cellular activity, by binding to critical cell surface glycoproteins, has been proposed before⁴⁴ and may prove to be one of the general functions of soluble animal lectins. A picture is therefore emerging that soluble lectins such as ϵ BP may be a broad-spectrum biological response modifier.

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References

- 1 Feizi, T. (1985) *Nature* 314, 53–69
- 2 Sharon, N. and Lis, H. (1989) *Science* 246, 227–246
- 3 Barondes, S.H. (1984) *Science* 223, 1259–1264
- 4 Leffler, H., Maslars, F.R. and Barondes, S.H. (1989) *Biochemistry* 28, 9222–9229
- 5 Liu, F-T. and Orida, N. (1984) *J. Biol. Chem.* 259, 10649–10652
- 6 Liu, F-T., Albrandt, K., Mendel, E., Ku'czycki, A., Jr and Orida, N.K. (1985) *Proc. Natl Acad. Sci. USA* 82, 4100–4104
- 7 Albrandt, K., Orida, N.K. and Liu, F-T. (1987) *Proc. Natl Acad. Sci. USA* 84, 6859–6863
- 8 Liu, F-T. (1990) *CRC Crit. Rev. Immunol.* 10, 289–306
- 9 Jia, S. and Wang, J.L. (1988) *J. Biol. Chem.* 263, 6009–6011
- 10 Laing, J.G., Robertson, M.W., Gritzmacher, C.A., Wang, J.L. and Liu, F-T. (1989) *J. Biol. Chem.* 264, 1907–1910
- 11 Sparrow, C.P., Leffler, H. and Barondes, S.H. (1987) *J. Biol. Chem.* 262, 7383–7390
- 12 Leffler, H. and Barondes, S.H. (1986) *J. Biol. Chem.* 261, 10119–10126
- 13 Raz, A., Pazerini, G. and Carmi, P. (1989) *Cancer Res.* 49, 3489–3493
- 14 Cherayil, B.J., Weiner, S.J. and Pillai, S. (1989) *J. Exp. Med.* 170, 1959–1972
- 15 Moutsatsos, I.K., Wade, M., Schindler, M. and Wang, J.L. (1987) *Proc. Natl Acad. Sci. USA* 84, 6452–6456
- 16 Wang, J.L., Werner, E.A., Laing, J.G. and Patterson, R.J. (1992) *Biochem. Soc. Trans.* 20, 269–274
- 17 Woo, H.J., Shaw, L.M., Messier, J.M. and Mercurio, A.M. (1990) *J. Biol. Chem.* 265, 7097–7099
- 18 Raz, A., Zhu, D., Hogan, V. et al. (1991) *Int. J. Cancer* 46, 871–877
- 19 Robertson, M.W., Albrandt, K., Keller, D. and Liu, F-T. (1990) *Biochemistry* 28, 8093–8100
- 20 Cherayil, B.J., Chaitovitz, S., Wong, C. and Pillai, S. (1990) *Proc. Natl Acad. Sci. USA* 87, 7324–7329
- 21 Oda, Y., Leffler, H., Sakakura, Y., Kasai, K. and Barondes, S.H. (1991) *Gene* 99, 279–283
- 22 Raz, A., Carmi, P., Raz, T. et al. (1991) *Cancer Res.* 51, 2173–2177
- 23 Drickamer, K. (1988) *J. Biol. Chem.* 263, 9557–9560
- 24 Springer, T.A. and Lasky, L.A. (1991) *Nature* 349, 196–197
- 25 Paroutaud, P., Levi, G., Teichberg, V.I. and Strosberg, A.D. (1987) *Proc. Natl Acad. Sci. USA* 84, 6345–6348

- 26 Abbott, W.M. and Feizi, T. (1991) *J. Biol. Chem.* 266, 5552-5557
- 27 Ohyama, Y. and Kasai, K. (1988) *J. Biochem.* 104, 173-177
- 28 Gitt, M.A., Massa, S.M., Leffler, H. and Barondes, S.H. (1992) *J. Biol. Chem.* 267, 10601-10606
- 29 Oda, Y., Herrmann, J., Gitt, M.A. *et al.* (1993) *J. Biol. Chem.* 268, 5929-5939
- 30 Gritzmacher, C.A., Mehl, V.S. and Liu, F-T. (1992) *Biochemistry* 31, 9533-9538
- 31 Cooper, D.N.W. and Barondes, S.H. (1990) *J. Cell Biol.* 110, 1681-1691
- 32 Frigeri, L.G. and Liu, F-T. (1992) *J. Immunol.* 148, 861-869
- 33 Frigeri, L.G., Robertson, M.W. and Liu, F-T. (1990) *J. Biol. Chem.* 265, 20763-20769
- 34 Clerch, L.B., Whitney, P., Hass, M. *et al.* (1988) *Biochemistry* 27, 692-699
- 35 Tracey, B.M., Feizi, T., Abbott, W.M. *et al.* (1992) *J. Biol. Chem.* 267, 10342-10347
- 36 Hsu, D.K., Zuberi, R. and Liu, F-T. (1992) *J. Biol. Chem.* 267, 14167-14174
- 37 Massa, S.M., Cooper, D.N.W., Leffler, H. and Barondes, S.H. (1993) *Biochemistry* 32, 260-267
- 38 Frigeri, L.G., Zuberi, R.I. and Liu, F-T. *Biochemistry* (in press)
- 39 Rosenberg, I., Cherayil, B.J., Isselbacher, K.J. and Pillai, S. (1991) *J. Biol. Chem.* 266, 18731-18736
- 40 Metzger, H., Alcaraz, G., Hohman, R. *et al.* (1986) *Annu. Rev. Immunol.* 4, 419-427
- 41 Brassart, D., Kolodziejczyk, E., Granato, D. *et al.* (1992) *Eur. J. Biochem.* 203, 393-399
- 42 Flotte, T.J., Springer, T.A. and Thorbecke, G.J. (1983) *Am. J. Pathol.* 111, 112-124
- 43 Truong, M-T., Gruart, V., Kusnierz, J-P. *et al.* (1993) *J. Exp. Med.* 177, 243-248
- 44 Feizi, T. and Childs, R.A. (1987) *Biochem. J.* 245, 1-11

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