Impairment of erythrocyte-antibody rosette formation by human lipocortin 1 on human leucocytes

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Lipocortins, a family of glucocorticoid-induced proteins of M, 33000–38000, inhibit phospholipase A, activity in vitro and have anti-inflammatory effects in animal models of inflammation. In view of a reported intra-membrane association between phospholipase A, and Fc, receptors on human leukaemic B-cell lines (Suzuki et al., 1980), we have examined the effect of lipocortin 1 binding to human mono-nuclear cells and neutrophils on the expression of Fc, receptors. Human lipocortin 1 has recently been cloned and expressed (Wallner et al., 1986) and the purified recombinant molecule used in these experiments was generously provided by the Biogen Research Corporation (Cambridge, MA, U.S.A.). Mononuclear cells or neutrophils were isolated from the peripheral blood of healthy subjects, washed in calcium-free phosphate-buffered saline to remove endogenous cells with increasing amounts of lipocortin. EA rosette formation by lipocortin 1 on human leucocytes

In a group of four subjects, incubation of mononuclear cells with increasing amounts of lipocortin 1 resulted in a dose-dependent inhibition of EA rosetting with maximum 70% inhibition occurring at 15 μg/ml (Fig. 1a). In a larger group of 12 individuals, incubation of mononuclear cells with this concentration of lipocortin resulted in a decrease in the median number of rosette-positive cells from 18.8 x 10⁴ (range 10.1-30.2 x 10⁴) to 5.5 x 10⁴ (range 0-14.5 x 10⁴), representing a significant fall of 70.8% (P<0.01) (Fig. 1b). A similar pattern of EA rosette inhibition was found for neutrophils, although maximum impairment of 60% occurred at 30 μg of lipocortin/ml. EA rosette inhibition did not occur when cells were incubated with sham preparations purified from Escherichia coli which did not contain the gene for human lipocortin. Also, the effect was abrogated when the preincubation step was carried out in calcium-free medium.

Abreviations used: EA, erythrocyte-antibody; mAb, monoclonal antibody.

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Fig. 1. Inhibition of EA rosette formation by lipocortin 1 on mononuclear cells (MNC)

(a) Reduction in EA rosette positivity after pretreatment of cells with increasing amounts of lipocortin (n = 4). (b) Reduction in EA rosette positivity after pretreatment of cells with 15 μg of lipocortin/ml (n = 12). Median bars and values shown. Level of significance P = 0.003 (Wilcoxon rank sum test).

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At a lipocortin concentration of 15 μg/ml there was no significant decrease in the expression of Fc, receptors as detected by mAbs directed against any of the three types of receptor on lymphocytes, monocytes or neutrophils (not shown).

In conclusion, this study suggests that lipocortin does not inhibit EA rosetting by a direct effect on receptor expression, but via an action on the mechanisms by which Fc, receptors associate within the membrane to form rosettes. Further studies are in progress to determine whether lipocortin is altering membrane fluidity generally or is binding at or in close proximity to the Fc, receptor, thereby affecting its lateral mobility within the plane of the membrane.

Characterization of the IgA receptor from human neutrophils

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IgA is the major immunoglobulin of sero-mucous secretions, where it defends external body surfaces. IgA is also a major serum protein, but in spite of a concentration equivalent to that of IgM or any of the subclasses of IgG, the biological functions of serum IgA remain unclear. Serum IgA in man is monomeric, whereas secretory IgA is polymeric containing in addition to IgA, two other proteins, J chain and secretory component. These cover the Fc region of the IgA molecule and therefore would be expected to alter any functions of IgA mediated by this region. Based largely on work with secretary IgA or IgA myelomas of unknown specificity, IgA has generally been assumed to be a poor activator of the complement system and to be unable to opsonize micro-organisms. The role of serum IgA in the inflammatory response is not known.

Serum IgA levels are frequently raised in rheumatic diseases and in other autoimmune diseases as well as in liver disease and persistent infections such as bacterial endocarditis. Elevated IgA levels are associated with rises in serum polymeric IgA and with IgA-containing immune complexes. Immune complexes containing IgA have been suggested to be pathogenic in these and other diseases such as the IgA nephropathies. In rheumatoid arthritis, IgA rheumatoid factor (IgA RF) has been shown to be present in the serum of the majority of patients, with IgA RF secreting lymphocytes being detectable in synovial fluid and at other sites of local inflammation. A strong correlation between raised IgA RF levels at the onset of disease and the eventual severity of the disease, has been reported.

In the past, the possibility that IgA might be involved in the pathogenicity of immune complex disease has been largely discounted because of its inefficiency in activating complement which was believed to be an important step in the generation of tissue damage. More recently, it has been realized that the activation of complement is in fact an essential step in the solubilization and removal of immune complexes. IgA-containing immune complexes, which activate complement poorly, could be more readily deposited in tissues and therefore be potentially more dangerous.

Abbreviations used: RF: rheumatoid factor; PMN, polymorphonuclear neutrophils.

Recently, we have shown that specific serum IgA antibodies are able to opsonize yeast for phagocytosis by polymorphonuclear neutrophils (PMN) in vitro, suggesting the presence of receptors for IgA on the neutrophil surface. Phagocytosis occurred in the absence of complement proteins and was more rapid than with IgG-opsonized yeasts (Yeaman & Kerr, 1987). We have now extended these studies to show that IgA anti-yeast mannann antibodies purified on Jacalin-Sepharose were able to trigger the phagocytosis of the yeast, confirming that IgA1 is opsonic for PMN. When phagocytosis assays were carried out in free solution, IgA was far more efficient as an opsonin than IgG, whereas with adherent PMN the two were of similar efficiency. Yeasts opsonized with either IgA or IgG elicited a chemiluminescent burst and caused the degradation of adherent PMN which could be measured by lysozyme release.

IgA anti-Escherichia coli antibodies isolated from a patient with liver disease and E. coli septicaemia were able to opsonize E. coli resulting in enzyme release and a chemiluminescent burst, when incubated with normal peripheral blood PMN. The bacteria were clearly phagocytosed when viewed in the electron microscope. In the case of both bacteria and yeasts opsonized with IgA, the chemiluminescent burst elicited from PMN was much more rapid than that elicited by complement-opsonized organisms.

We have also shown that IgA-Sepharose or IgG-Sepharose, but not bovine serum albumin-Sepharose, was far more efficient than IgG-Sepharose as an opsonin for E. coli resulting in enzyme release and a chemiluminescent burst, when incubated with normal peripheral blood PMN. The bacteria were clearly phagocytosed when viewed in the electron microscope. In the case of both bacteria and yeasts opsonized with IgA, the chemiluminescent burst elicited from PMN was much more rapid than that elicited by complement-opsonized organisms.

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