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Association between pregnancy-associated α_2-glycoprotein (α_2-PAG) and mixed leucocyte reaction determinants on the leucocyte surface

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Summary. α_2-PAG is present on the surface of mononuclear blood leucocytes and can be demonstrated predominantly on B-lymphocytes and monocytes. Pretreatment of cells with antibody to α_2-PAG leads to a marked reduction in Fc-rosette formation. Competitive blocking experiments with specific antisera reveal a particularly close association between α_2-PAG and MLR (mixed leucocyte reaction) determinants on the cell surface. These findings suggest one mechanism whereby α_2-PAG may modify cell-mediated immune responses.

Modification of cell-mediated immunity by factors present in serum is thought to play some part in tolerance of the pregnant female towards her foetus[1-4]. Prominent among the substances to which such an effect has been attributed is pregnancy-associated α_2-glycoprotein (α_2-PAG) a macroglobulin of mol. wt about 300,000[5], present, in trace amounts, in all normal sera[6,7] and which appears to be a leucocyte product[8]. The serum level of this protein rises dramatically during pregnancy[5,9] and raised levels have also been reported in patients with disseminated malignancies[10-12]. It has apparent immunosuppressive properties in vitro, i.e. reduces antigen and phytomitogen-induced lymphocyte transformation[13,14], blocks the mixed leucocyte reaction (MLR)[15] and impairs macrophage electrophoretic mobility[16].

To understand the mechanism whereby α_2-PAG exerts its influence on cellular immune reactions, it is essential to determine the distribution of the protein on human leucocytes and its relationship to cell surface determinants already characterized. Recent studies have shown that α_2-PAG can be identified on human B-lymphocytes and monocytes[17,18]. These cells are also known to carry the surface determinants (HLA-D) responsible for stimulation in mixed leucocyte cultures[19,20]. There is a close spatial relationship between serologically determined HLA-D related antigens (the 'DRW' series, formerly 'Ia') and the Fc receptor site on B-lymphocytes[21]. An association between α_2-PAG and the products of the HLA-DR locus is therefore suggested by the demonstration that pretreatment of mononuclear cells with anti-α_2-PAG antibody markedly reduces the number of Fc-rosette-forming cells[17].

In the present study we have extended these observations and have compared the distribution of α_2-PAG and other surface determinants on human leucocytes.

Materials and methods. Mononuclear cells were obtained from 10-ml samples of EDTA-treated peripheral blood by centrifugation on Ficoll-Triosil[22]. 'E'-rosettes were formed by incubating the cells overnight at 4 °C with 2-aminoethylisothiouronium bromide-treated sheep erythrocytes[23]. Rosetting and nonrosetting cells were then separated by repeating the centrifugation on Ficoll-Triosil. Red cells were removed by osmotic lysis.

Direct and indirect immunofluorescence staining for α_2-PAG were carried out as previously described[17,18] using a commercial rabbit anti-α_2-PAG IgG (Dakopatts) and goat antirabbit gamma globulin (Nordic). Fc-receptor-bearing cells were demonstrated by rosette-formation with IgG-coated ox erythrocytes[24,25]. The inhibitory effect of anti-α_2-PAG on Fc-rosette formation was demonstrated using either intact IgG or F (ab')$_2$ fragments of the antibody[26].

The following commercially-available rabbit antisera to human cell surface determinants were used: from Dakopatts, anti-IgM (μ-chain specific) and anti-β_2 microglobulin (β_2M); from Sera-Lab, anti-HLA (all specificities) and anti-Ia ('DRW'). In addition, rabbit antisera were raised against partially-purified surface membranes of human B lymphoblastoid cell lines. One of the lines used was EB_1, which carries HLA, β_2M and 'DRW' determinants but which does not stimulate in mixed leucocyte culture and hence appears to lack the MLR antigen product of the HLA-D locus[27]. Anti-EB_1 serum was extensively absorbed with T-cell lines ($MOLT_4$ and CCRF-CEM) before use. In microcytotoxicity and indirect immunofluorescence tests this antiserum behaves like an anti-DRW reagent i.e. it is highly reactive with all B-cell lines and with peripheral blood B-cells but not with T-cells. Rabbit antiserum was also raised to membranes from a second B-cell line, DAUDI. This line does not produce β_2M[28] and hence does not carry surface HLA, A-, B- or C-determinants. It does express DRW antigens and is a potent stimulator in mixed leucocyte cultures[27]. Anti-DAUDI serum was extensively absorbed with T-cell lines and with EB_1 cells in an attempt to derive a reagent which is specific for MLR determinants. In microcytotoxicity assay the resultant 'anti-MLR' serum is relatively weakly reactive with peripheral blood B-cells and with all B-cell lines (except EB_1, with which it is unreactive[29]). A similar level of activity is observed on indirect immunofluorescence testing; yet at greater dilutions the antiserum retains MLR-blocking activity while, unlike anti-DRW reagents, it does not inhibit mitogen-induced lymphocyte activation[29]. The Student t-test was used for statistical analysis of results.

Results and discussion. Table 1 shows that α_2-PAG can be identified on the surface membranes of mononuclear cells and that it is predominantly associated with those cells which do not form 'E'-rosettes (non-T-cells). Pretreatment with unlabelled specific antibody to α_2-PAG resulted in a 20% reduction ($p < 0.025$), with respect to the control value,

Table 1. Incidence of α_2-PAG positive 'E'-rosetting and 'non-E' cells in peripheral blood of normal individuals*

Cells	α_2-PAG positive (%)
E-rosetting	6.0± 3.0
'non-E'	76.4± 10.4

Results (mean± 1 SD) based on samples from 6 individuals. *Unseparated mononuclear leucocytes from 22 normal donors (including the above 6) contain 24± 11.6% α_2-PAG +ve cells[18].

Table 2. Effect of pretreatment of mononuclear cells with anti-α_2-PAG on subsequent rosette formation

Rosettes	No. of donors	Rosetting cells (%) Untreated	Anti-α_2-PAG IgG	Anti-α_2-PAG F (ab')$_2$
E	6	55.4± 5.4	44.4± 7.0	ND
Fc	6	15.1± 4.3	7.8± 2.6	ND
	3	14.3± 6.0	6.4± 3.6	5.3± 2.0
C3	6	19.7± 7.0	21.3± 8.0	ND

Results are means± 1 SD. ND: not done

Table 3. Effect of pretreatment of mononuclear cells with various antisera on the incidence of α_2-PAG positive cells

Antiserum	α_2-PAG positive cells (%) Donor1	Donor2	Inhibition (%) Donor1	Donor2
Untreated	27.9	16.0	0	0
HLA (all specificities)	12.8	ND	54	ND
β_2-microglobulin	14.9	11.2	47	30
IgM (μ-chain-specific)	14.1	ND	50	ND
DRW_a	4.9	6.2	82	62
DRW_b	7.2	5.5	74	66
MLR	1.4	3.7	95	77
Control (anti-MLR absorbed with DAUDI cells)	ND	15	ND	6

DRW_a: Rabbit antiserum to 'Ia' antigens (Sera-Lab). DRW_b: Rabbit antiserum to EB_1 membranes absorbed with T-cells ($MOLT_4$+CCRF-CEM). MLR: Rabbit antiserum to DAUDI membranes absorbed with T-cell lines and EB_1. * Based on scoring of at least 200 cells from each sample. In both experiments the observer did not know the arrangement of test or controls. ND: not done.

in the incidence of E-rosette forming cells (table 1). However, a more pronounced reduction (50%, $p < 0.0125$) relative to controls was found in the incidence of Fc-rosette forming cells. No effect was observed on C3-rosette formation.

In 2 separate experiments, pretreatment of mononuclear cells with a variety of specific antisera to human cell surface antigens caused partial blocking of subsequent staining for α_2-PAG (table 3). Although blocking was obtained with each antiserum, the maximal effect in both experiments followed pretreatment with anti-MLR serum.

The immunofluorescence and rosette inhibition data confirm that α_2-PAG is mainly associated with the surface membranes of B-cells and monocytes. Blocking of α_2-PAG staining by anti-μ-chain, anti-β_2M and anti-HLA sera may be nonspecific (in the sense that spatial association of all determinants on the surface of a given cell is inevitable) but the maximal reduction of staining which follows pretreatment with anti-MLR antibody suggests a particularly close association of α_2-PAG with MLR antigens on B-lymphocytes (and probably also on monocytes).

There is not likely to be significant cross-reactivity between the antisera to α_2-PAG and MLR for the following reasons. 1. Anti-α_2-PAG does *not* react with DAUDI, or other B-cell lines, which do express MLR determinants, in indirect immunofluorescence or microcytotoxicity tests. 2. Blocking activity of anti-MLR serum is removed by a single absorption with DAUDI. 3. In preliminary studies which form the basis of a separate report, anti-α_2-PAG inhibits mitogen-induced lymphocyte activation as well as MLR. In the same experiments anti-MLR serum, as previously demonstrated[29], inhibits MLR only.

It is now established that α_2-PAG can be synthesized by oestrogen-stimulated leucocytes[17] and that in pregnancy there is a marked increase in plasma α_2-PAG concentrations[5,9]. Both pregnancy plasma[1-4] and α_2-PAG suppress MLR[15] and hence α_2-PAG may have an important role in vivo in suppression of foetal allograft rejection. In this context, it is perhaps relevant that a low plasma level of α_2-PAG in early pregnancy is associated with an increased risk of spontaneous abortion[30].

The immunosuppressive effect of α_2-PAG can be related to its close physical association with MLR determinants by postulating that, in the complex so formed, MLR antigens are masked or modified, thus substantially reducing the stimulus which would otherwise set in train a cell-mediated anti-allograft response.

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