Clin. exp. Immunol. (1981) **46**, 350–354.

Inhibition of human monocyte Fc receptor and HLA-DR antigen expression by pregnancy alpha-2 glycoprotein

R. H. PERSELLIN & J. RHODES *Strangeways Research Laboratory and Immunology Division, Department of Pathology, University of Cambridge, UK, and University of Texas Health Science Center, San Antonio, Texas, USA*

(Accepted for publication 1 May 1981)

SUMMARY

The precise factor responsible for the altered immunological reactivity and diminished inflammatory responses occurring in pregnancy is unknown. Recent observations implicate the pregnancy-associated alpha-2 glycoprotein (PAG). Using rosette assays for the detection of cells carrying Fc receptors and for the demonstration of surface HLA-DR antigens, we demonstrate that a serum protein fraction rich in PAG inhibits these surface markers on human monocytes. Brief exposure of normal peripheral blood monocytes to physiological concentrations of PAG led to almost total loss of Fc receptor expression, an effect that was concentration-related. Using a monoclonal anti-HLA-DR antibody linked to red cells, physiological concentrations of PAG also significantly inhibited the detection of HLA-DR on monocytes. As controls, we used a protein fraction isolated in identical fashion from male sera devoid of PAG, and an alpha-2 macroglobulin isolate. These findings suggest that PAG, by masking monocyte surface markers, could be responsible for the suppression of some cell functions during pregnancy.

INTRODUCTION

Some inflammatory and immunologically mediated disorders subside during pregnancy. Theories to explain these observations as well as the failure of the mother to reject the fetus during pregnancy have been the subject of recent reviews (Persellin, 1977; Beer & Billingham, 1978; Stites *et al.*, 1979). Many investigators have attributed the diminished cellular immunity as well as the inhibition of both polymorphonuclear leucocyte (PMN) and macrophage functions detected during gestation to a soluble factor in pregnancy serum. Although the precise factor(s) responsible for these *in vitro* observations is not known, recent studies have focused on the role of the pregnancy-associated alpha-2 glycoprotein (PAG) (von Schoultz, Stigbrand & Tarnvik, 1973; Than *et al.*, 1975; Stimson, 1976; Persellin & Leibfarth, 1978; Persellin & Thorne, 1981). In this paper we present evidence that a PAG-rich fraction derived from pregnancy serum masks both Fc receptor expression and HLA-DR antigen detection on the surface of human monocytes. These findings could explain the inhibition of receptor-dependent cell functions by pregnancy serum and suggest a mechanism for the subsidence of inflammatory and immunological disorders during gestation.

MATERIALS AND METHODS

PAG isolation. Pregnancy sera were obtained from human donors in their 32nd week of uncomplicated gestation and tested for the presence of PAG. Those with high concentrations were pooled; each pool was derived from 10 to 16 donors. An age-matched normal male serum pool

Correspondence: Dr R. H. Persellin, Division of Rheumatology, Department of Medicine, University of Texas Health Science Center, San Antonio, Texas 78284, USA.

0009-9104/81/1100-0350$02.00

without detectable PAG was used as a control and handled identically. A PAG-enriched fraction was isolated (Persellin & Starkey, in preparation) using polyethylene glycol precipitation, Sephacryl S-300 gel chromatography and DEAE gradient separation. Following this stage of isolation, both the pregnancy and the male fractions contained alpha-2 macroglobulin (α_2M) and small amounts of haptoglobin. Neither fraction contained immunoglobulins. The concentration of PAG/mg total protein in the pregnancy fraction was 120-times enriched with respect to the starting serum pool. The pregnancy isolate contained 2,079 μg protein/ml of which 216 μg/ml was PAG, 1,850 μg/ml was α_2M and less than 50 μg/ml was haptoglobin. The male serum-derived fraction had 2,045 μg protein/ml of which 2,000 μg/ml was α_2M and less than 50 μg/ml was haptoglobin. PAG was not detected in the male fraction. Thus any differences in the effects of these preparations on cell surface marker expression could be attributed to PAG. An α_2M isolated (kindly provided by Dr A. J. Barrett) was studied at identical concentrations. All protein solutions were dialysed exhaustively against Hanks' balanced salt solution (HBSS) and filtered through 0·22-μm Millipore membranes prior to study. Serum proteins were quantified using the method of Lowry *et al.* (1951), and the rocket immunoassay technique of Laurell (1972). The purity of isolates was determined by polyacrylamide and agarose electrophoretic methods. Antiserum to PAG (provided by Dr B. H. Berne) was prepared by immunizing a male goat with the alpha-2 macroglobulin fraction isolated from pooled normal human pregnancy sera and absorbing the resulting immune serum with normal male serum. The absorbed antiserum was monospecific for PAG and did not react with either male, non-gravid female or cord sera. A monospecific antiserum to α_2M was raised in rabbits in the laboratory of Dr A. J. Barrett.

Monocytes. Normal human mononuclear cells were isolated from defibrinated blood by centrifugation over a Ficoll–Triosil gradient as previously described (Rhodes, Bishop & Benfield, 1979). Cells were washed twice in HBSS and suspended at a concentration of 2×10^6/ml in RPMI 1640 containing 20% heat-inactivated fetal calf serum. One millilitre was placed in each chamber of dual tissue culture slides (Lab-Tek Products), incubated at 37°C for 1 hr and then washed with serum-free medium to remove non-adherent cells. Approximately 95% of the adherent cells at this stage are monocytes.

Fc receptor expression. This assay was performed using ox erythrocytes sensitized with heat-inactivated rabbit anti-ox antiserum as previously described (Rhodes, 1977). The binding of this reagent reflects the expression of Fc receptors since it is specifically inhibited by heat-aggregated human IgG. Briefly, a 1% suspension of erythrocytes was incubated with a dilution of antiserum that would permit detection of Fc receptors on 80–90% of human adherent monocytes. Rosette-forming cells were quantified after incubation for 30 min at 20°C with 1 ml of a 1% suspension of the sensitized erythrocytes followed by washing and staining of the tissue culture slides. Cells with three or more attached erythrocytes were scored as rosettes.

HLA-DR antigen detection. This membrane component was examined by means of a monoclonal antibody specific for a determinant in the non-polymorphic region of the HLA-DR product (Brickell, McConnell & Milstein, 1981). This reagent was used in a red cell-linked assay (Coombs *et al.*, 1977) with which the antigen was detectable on more than 80% of peripheral blood monocytes. Following brief exposure to the PAG-containing or control serum fractions, cells were washed three times in cold HBSS before the rosette assay was performed. Heat-aggregated human IgG, which will inhibit monocyte Fc rosettes, did not inhibit the detection of HLA-DR rosettes (Rhodes & Stokes, unpublished observations).

The serum protein fractions were each studied at two dilutions, 400 and 2,000 μg/ml. Adherent monocytes were incubated with the PAG-containing or control solutions for 30 min at 37°C and then washed with HBSS without protein prior to the addition of sensitized erythrocytes. Several replicate slides were prepared for each variable studied, coded and then counted as unknowns by two observers. Approximately 500 monocytes were scored on each slide. Student's *t*-test was used to determine probabilities.

RESULTS

When preincubated in HBSS buffer alone, 86% of the adherent monocytes formed rosettes with the

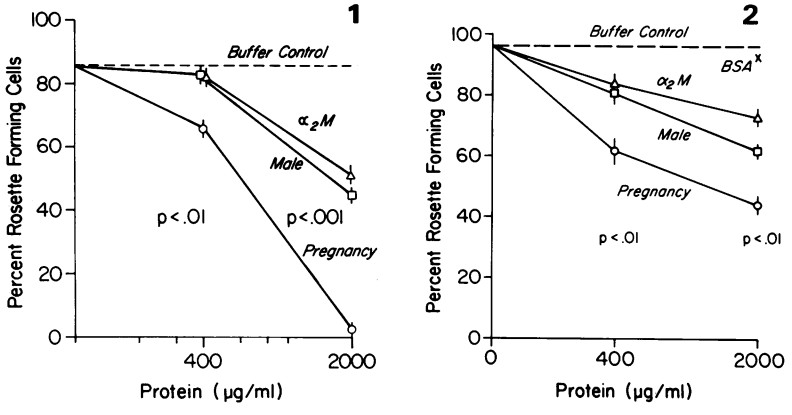

Fig. 1. Effect of serum protein fractions on human peripheral blood monocyte Fc receptor expression. All protein fractions were derived from human serum pools by polyethylene glycol precipitation and column chromatography methods. Following exhaustive dialysis in buffer (HBSS) each fraction was studied at identical total protein concentrations of 400 and 2,000 μg/ml. Shown are the means ± 1 s.e.m. for four replicates counted by two observers. See Results for details.

Fig. 2. The detection of an HLA-DR antigen on human monocyte membranes using a monoclonal anti-HLA-DR-linked red cell rosette assay. Protein fractions studied were identical to those used in Fc receptor experiments. See Results for details. The probabilities expressed contrast pregnancy with the male fractions.

sensitized ox erythrocytes. This degree of Fc receptor expression was not changed if the cells were preincubated in bovine serum albumin, 2,000 μg/ml. However, as shown in Fig. 1, fewer cells formed rosettes when preincubation was with either α_2M or the male serum fraction (in which the major protein was α_2M) at 2,000 μg/ml. Alpha-2 macroglobulin was also the major component of the pregnancy serum-derived fraction but the latter contained, in addition, PAG at physiological concentrations. This fraction at a protein concentration of 2,000 μg/ml almost totally inhibited Fc receptor expression. The difference between the pregnancy and male serum fractions was highly significant ($P < 0{\cdot}001$). This statistically significant difference was maintained even when the isolates were studied at concentrations of 400 μg/ml. Although both the α_2M and the male isolates no longer differed from the HBSS control, the pregnancy fraction significantly inhibited rosetting. Thus physiological concentrations of PAG were shown to decrease macrophage Fc receptor expression.

As a step towards determining the degree of specificity exhibited by this effect, the expression of another monocyte membrane component, distinct from the Fc receptor, was examined. As shown in Fig. 2, PAG prevented HLA-DR antigen detection. Significantly fewer rosettes were present when monocytes were preincubated with the pregnancy serum-derived fraction. This fraction, studied at a total protein concentration of 400 μg/ml, contained 42 μg PAG/ml, a level observed after the first trimester in most pregnancies. Increasing the concentration further decreased the percentage of rosette-forming cells, though total inhibition was not observed. As further documentation of PAG effect, Fc receptor assays were performed simultaneously on the same population of normal monocytes used to study HLA-DR. Results closely reproduced those presented in Fig. 1.

DISCUSSION

One explanation for the failure to reject the fetus and for the subsidence of inflammatory disorders during pregnancy is that pregnancy serum contains factors that suppress leucocyte functions. In support of this are studies showing that both antigen- and mitogen-induced lymphocyte transformation and the mixed lymphocyte culture reaction are diminished by pregnancy serum

(Kasakura, 1971; Leikin, 1972; Purtilo, Hallgren & Yunis, 1972; Fujisaki *et al.*, 1979). In addition to suppressing the function of mononuclear cells, pregnancy serum also inhibits polymorphonuclear leucocytes (PMN) functions (Persellin & Leibfarth, 1978; Bjorksten *et al.*, 1978; Takeuchi & Persellin, 1980; Persellin & Thorne, 1981). In these studies, washed control or gestational PMN in non-gravid serum functioned normally; but cells suspended in pregnancy serum were less efficient in ingesting a variety of particles and also exhibited a decreased chemotactic response to endotoxin-activated serum. This wide range of inhibitory actions on different cells by a soluble factor in pregnancy serum suggested to us that the substance responsible might act by masking surface receptors.

Numerous constituents of pregnancy serum have been implicated, including steroid and tropic hormones, α-fetoprotein, immune complexes and the pregnancy-related serum proteins (Persellin, 1977; Beer & Billingham, 1978; Stites *et al.*, 1979). Whichever is the putative inhibitor, its appearance and concentration in pregnancy bloods should parallel the clinical course of rheumatoid arthritis during gestation (Neely & Persellin, 1977).

In this paper, we demonstrate that PAG could be responsible for these diverse *in vitro* actions of pregnancy serum. PAG is a glycoprotein with a molecular weight of 364,000 daltons and is present in the serum in concentrations greater than 500 μg/ml in the third trimester of 78% of pregnancies. The amount in blood increases during the first trimester and remains elevated throughout gestation, falling to pre-gravid levels 6 weeks post-partum. Thus concentrations of PAG in the blood closely parallel the time course of development of both the *in vitro* effects of pregnancy serum on cell functions and the clinical course of inflammatory and immunologically-mediated disorders during gestation (Persellin, 1977). The exact role of PAG is uncertain but it has been identified on the surface membranes of human peripheral blood lymphocytes (Stimson, 1977) and monocytes (Thomson *et al.*, 1979).

We believe the dramatic inhibition of Fc receptor expression on human peripheral blood monocytes by the pregnancy serum fraction is due to physiological concentrations of PAG. This isolate differed from the male serum fraction only in the presence of PAG. The mild degree of inhibition of rosetting seen here with the male fraction at 2,000 μg/ml is likely to be due to the presence of α_2M in this fraction. But for trace amounts of haptoglobin, the male serum isolate contained only α_2M. Isolated α_2M at the same concentration exhibited an identical degree of rosetting inhibition. This finding is consistent with some previous reports of the effects of α_2M on the immune system as reviewed by James (1980). However, when studied at 400 μg/ml, neither the α_2M nor the male fraction was inhibitory, whereas the pregnancy isolate was.

Inhibition of HLA-DR detection is further evidence of PAG interaction with the monocyte surface. Interestingly, the almost total inhibition of Fc rosetting observed with the higher PAG concentration was not seen in the HLA-DR rosette assay. Direct inhibition studies show that the Fc receptor and the DR structure are distinct on the monocyte membrane and that there is no obligatory close association between the two (Rhodes & Stokes, unpublished observations). Nevertheless, some DR antigens are likely to be closer to Fc receptors than others and the probability of a DR antigen being masked by surface-bound PAG may be inversely related to its distance from the Fc receptor. This would explain the lack of total masking of HLA-DR antigens by PAG at the higher concentration.

The previous observations with PAG taken together with our findings presented here suggest that PAG acts at the cell surface. Impairment of leucocyte function in the presence of physiological concentrations of PAG could explain the altered immunoregulatory function and diminished inflammatory reactions observed during pregnancy.

The authors thank Professor Charles P. Douglas, Cambridge, for providing pregnancy serum, Dr B. H. Berne, Washington, DC, for antiserum to PAG, Mr P. Brickell and Dr I. McConnell for monoclonal anti-HLA-DR antibody, and Drs B. von Schoultz, Umea, Sweden, W. H. Stimson, Glasgow, and C. H. W. Horne, Aberdeen, for PAG standard measurements.

This study was supported in part by grants from the Robert A. Welch Foundation of Houston, Texas, the South Central Texas Chapter of the Arthritis Foundation, the Cancer Research Campaign, London, and by an Arthritis Clinical Research Center grant from the Arthritis Foundation.

REFERENCES

BEER, A.E. & BILLINGHAM, R.E. (1978) Immunoregulatory aspects of pregnancy. *Fed. Proc.* **37,** 2374.

BJORKSTEN, B., SODERSTROM, T., DAMBER, M.G., VON SCHOULTZ, B. & STIGBRAND, T. (1978) Polymorphonuclear leucocyte function during pregnancy. *Scand. J. Immunol.* **8,** 257.

COOMBS, R.R.A., WILSON, A.B., EREMIN, O., GURNER, B.W., HAEGERT, D.G., LAWSON, Y.A., BRIGHT, S. & MUNRO, A.J. (1977) Comparison of the direct antiglobulin rosetting reaction with the mixed antiglobulin rosetting reaction for the detection of immunoglobulin on lymphocytes. *J. Immunol. Methods*, **18,** 45.

BRICKELL, P., MCCONNELL, I. & MILSTEIN, C. (1981) A monoclonal antibody to the HLA-DR product recognizes a polymorphic Ia determinant in mice. *Immunology*. (In press.)

FUJISAKI, S., MORI, N., SASAKI, T. & MAEYAMA, M. (1979) Cell-mediated immunity in human pregnancy. Changes in lymphocyte reactivity during pregnancy and post-partum. *Microbiol. Immunol.* **23,** 899.

JAMES, K. (1980) Alpha-2 macroglobulin and its possible importance in immune systems. *Trends Biochem. Sci.* **5,** 43.

KASAKURA, S. (1971) A factor in maternal plasma during pregnancy that suppresses the reactivity of mixed leukocyte cultures. *J. Immunol.* **107,** 1296.

LAURELL, C.B. (1972) Electroimmunoassay. *Scand. J. clin. Lab. Invest.* **29,** 21.

LEIKIN, S. (1972) The immunosuppressive effect of maternal plasma. In *Proceedings of the Sixth Leukocyte Culture Conference, New York*, p. 725. Academic Press, New York.

LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. & RANDAL, R.J. (1951) Protein measurement with Folin phenol reagent. *J. biol. Chem.* **193,** 265.

NEELY, N.T. & PERSELLIN, R.H. (1977) Activity of rheumatoid arthritis during pregnancy. *Tex. Med.* **73,** 59.

PERSELLIN, R.H. (1977) The effect of pregnancy on the rheumatic diseases. *Bull. Rheum. Dis.* **27,** 922.

PERSELLIN, R.H. & LEIBFARTH, J.K. (1978) Studies of the effects of pregnancy serum on polymorphonuclear leukocyte functions. *Arthritis Rheum.* **21,** 316.

PERSELLIN, R.H. & THORNE, K.J.I. (1981) Inhibition of phagocytosis of *Trypanosoma dionisii* by pregnancy alpha-2 glycoprotein. *Trans. R. Soc. Trop. Med. Hyg.* **75,** 436.

PURTILO, D.T., HALLGREN, H.M. & YUNIS, E.J. (1972) Depressed maternal lymphocyte response to phytohaemagglutinin in human pregnancy. *Lancet*, **i,** 769.

RHODES, J. (1977) Altered expression of human monocyte Fc receptors in malignant disease. *Nature*, **265,** 253.

RHODES, J., BISHOP, M. & BENFIELD, J. (1979) Tumor surveillance: how tumors may resist macrophage-mediated host defense. *Science*, **203,** 179.

STIMSON, W.H. (1976) Studies on the immunosuppressive properties of a pregnancy-associated α-macroglobulin. *Clin. exp. Immunol.* **25,** 199.

STIMSON, W.H. (1977) Identification of pregnancy-associated α-macroglobulin on the surface of peripheral blood leucocyte populations. *Clin. exp. Immunol.* **28,** 445.

STITES, D.P., PAVIA, C.S., CLEMENS, L.E., KUHN, R.W. & SIITERI, P.K. (1979) Immunologic regulation in pregnancy. *Arthritis Rheum.* **22,** 1300.

THAN, G.N., CSABA, I.F., KARG, N.J., SZABO, D.G., AMBRUS, M. & BAJTAI, G. (1975) Immunosuppressive effect of pregnancy-associated alpha$_2$-glycoprotein. *Lancet*, **ii,** 515.

TAKEUCHI, A. & PERSELLIN, R.H. (1980) The inhibitory effect of pregnancy serum on polymorphonuclear leukocyte chemotaxis. *J. clin. Lab. Immunol.* **3,** 121.

THOMSON, A.W., HUNTER, C.B.J., CRUICKSHANK, N. & HORNE, C.H.W. (1979) Study of pregnancy-associated alpha$_2$-glycoprotein in relation to populations of human blood leucocytes. *Int. Arch. Allergy appl. Immunol.* **58,** 251.

VON SCHOULTZ, B., STIGBRAND, T. & TARNVIK, A. (1973) Inhibition of PHA-induced lymphocyte stimulation by the pregnancy zone protein. *FEBS Lett.* **38,** 23.