Homocytotropic (IgE) antibody titers in pregnant and non-pregnant rats immunized with non-dialyzable water soluble ragweed (DWSR) antigen

Pregnant rat number	PCA titer	Non-pregnant rat number	PCA titer
1	32	11	256
2	64	12	256
3	128	13	0
4	256	14	64
5	0	15	128
6	16	16	64
7	16	17	8
8	256	18	16
9	256	19	256
10	16		

100 µg DWSR precipitated with 30 mg of aluminum hydroxide gel Al(OH), and the other group was given an equal amount of vehicle through the same route. Similarly, 2 groups of non-pregnant females (9 animals per group) were given either a single i.p. injection of $Al(OH)_3$ + DWSR or $Al(OH)_3$ alone. 10 days post immunization, all animals were anaesthetized with ether, and blood was collected from the abdominal aorta near the iliac bifurcation. The blood was allowed to clot in ice-chilled water and the serum was separated by centrifugation. Aliquots of the serum from each rat were stored at –70 °C until analyzed.

The level of circulating antibody (IgE) was estimated by passive cutaneous anaphylaxis (PCA) test[5] with a sensitization period of 48 h. Thus, 0.1 ml quantities of saline dilutions of test serum were injected intradermally into the recipient male outbred Wistar rats (Woodlyn Farms) and each injection was duplicated on different animals. All rats were injected i.v. 48 h later with 0.2 ml of short ragweed (1:20) mixed with 0.8 ml of 2% Evans blue dye. The skin reactions were examined after 30 min. The antibody titers were expressed as the reciprocal of the highest dilution giving the diameter of blueing reaction greater than 5 mm.

Results and discussion. The table shows that there is no apparent difference between the homocytotropic (IgE) antibody titer in the sera of pregnant and non-pregnant rats immunized with DWSR. In a separate experiment, no PCA reaction was detected in the sera of pregnant and non-pregnant rats injected with $Al(OH)_3$ alone. The data suggest that there is no evidence of immunosuppressive factor in humoral response during pregnancy. It is very well-known that proteins are T-dependent antigens[6,7], and therefore, if there is any suppression of T-cells[1,2], one would expect a lower humoral response in pregnant rats compared with the non-pregnant ones. It is quite likely that the factor(s), secreted by T-cells responsible for B-cell stimulation[8], are not suppressed during pregnancy. However, it would be interesting to evaluate humoral response with a number of antigens in different species at different intervals of pregnancy.

5 Z. Ovary, in: Immunological Methods, p. 259. Ed. J. F. Ackroyd. Blackwell Scientific Publication, Oxford 1964.
6 T. Kishimoto and K. Ishizaka, J. Immun. *109*, 612 (1972).
7 T. Tada, K. Okumura and M. Taniguchi, J. Immun. *108*, 1535 (1972).
8 R. H. Gisler, F. Staber, E. Rüde and P. Dukor, Eur. J. Immun. *3*, 650 (1973).

Species difference in the effects of proteolytic enzymes on red cell membrane

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Summary. Pronase and α-chymotrypsin digested the major glycoprotein in the human and mouse red cell membranes and in SDS gel electrophoresis the glycoprotein disappeared accompanied by the appearance of a new band of lower mol.wt. However in the membranes of sheep, rat and rabbit, no digestion was demonstrated. The effects of pronase on anion permeability were almost identical for human and animal erythrocytes.

The outer suface of blood cells is rich in carbohydrate. This glycoprotein layer has been implicated in the immunological and physiological characteristics of these cells. Pronase degrades the glycoprotein on the outer surface of the human erythrocyte membrane which runs in SDS polyacrylamide disc gel electrophoresis a at mol.wt of 93,000. Carbohydrate and sialic acid are removed but fragments of mol.wt 61,000 remains attached to the membrane[1]. Pronase produces several effects on the cell membrane, including the digestion of glycoprotein, the inhibition of anion permeability and increase in cation permeability[2]. The α-chymotrypsin attack on the glycoprotein is similar to that of pronase, but the proteolysis is less vigorous[3]. The effects of the enzymes on animal erythrocytes were studied by SDS polyacrylamide disc gel electrophoresis and anion permeability.

Materials and methods. All experiments were performed with heparinized blood from rat, mouse, sheep, rabbit and human. Red blood cells were washed three times with isotonic NaCl-Tris pH 7.4. Red cells were incubated with pronase (0.2 mg/ml, Kaken Kagaku) and α-chymotrypsin (2 mg/ml, Boehringer, Mannheim, BRD) in isotonic NaCl-Tris pH 7.4 at a hematocrit of 30% at 37 °C for 30 min with gentle shaking. The suspensions were washed 6 times with ice cold NaCl-Tris. Red cell ghosts were prepared by the method of Dodge et al[4]. Solubilization of each membrane prior to electrophoresis was achieved by the addition of 0.5 ml of the solution of 3%

1 W. W. Bender, H. Green and H. C. Berg, J. molec. Biol. *58*, 783 (1971).
2 H. Passow, J. Membrane Biol. *6*, 233 (1971).
3 Z. I. Cabantchik and A. Rothstein, J. Membrane Biol. *15*, 227 (1974).
4 J. T. Dodge, C. Mitchell and D. Hanahan, Archs Biochem. Biophys. *110*, 119 (1963).

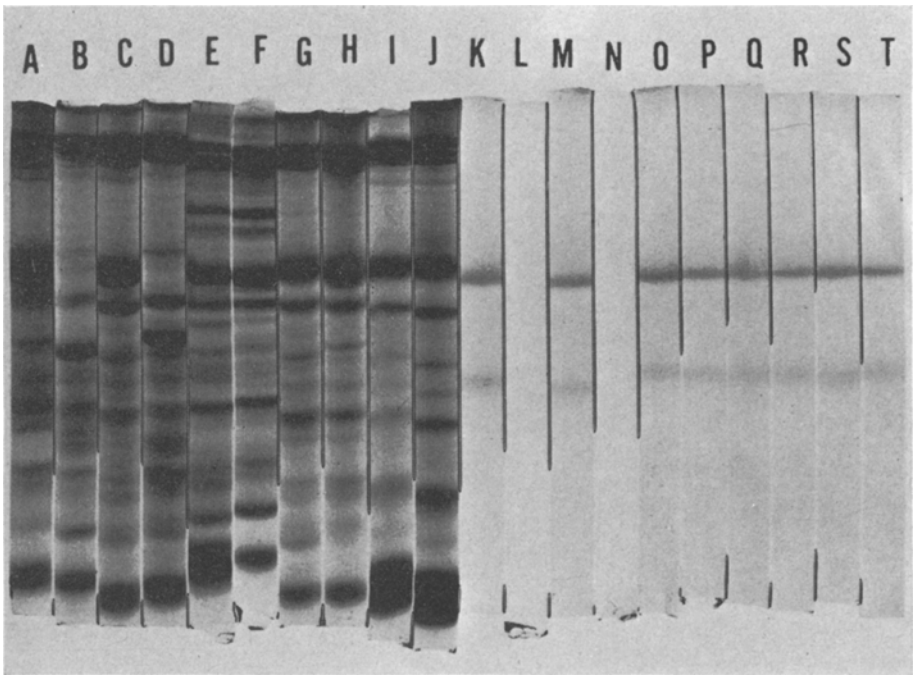

Fig. 1. Effects of pronase on animal red cells in SDS polyacrylamide disc gel electrophoresis. *A–J* Coomassie blue stain; *K–T* PAS stain. *A*, *K* Human, control; *B*, *L* human, pronase; *C*, *M* mouse, control; *D*, *N* mouse, pronase; *E*, *O* sheep, control; *F*, *P* sheep, pronase; *G*, *Q* rabbit, control; *H*, *R* rabbit, pronase; *I*, *S* rat, control; *J*, *T* rat, pronase.

SDS, 0.1% mercapto-ethanol, 5 M urea, 1 mM EDTA and 0.1 M Tris-HCl pH 7.6 to 0.5 ml of the membrane suspension, and the mixture was heated at 60 °C for 3 min. SDS polyacrylamide disc gel electrophoresis was prepared, run and stained according to the methods of Fairbanks et al[5]. Gels were stained for protein with Coomassie blue and for carbohydrate with periodic acid-Schiff reagent (PAS).

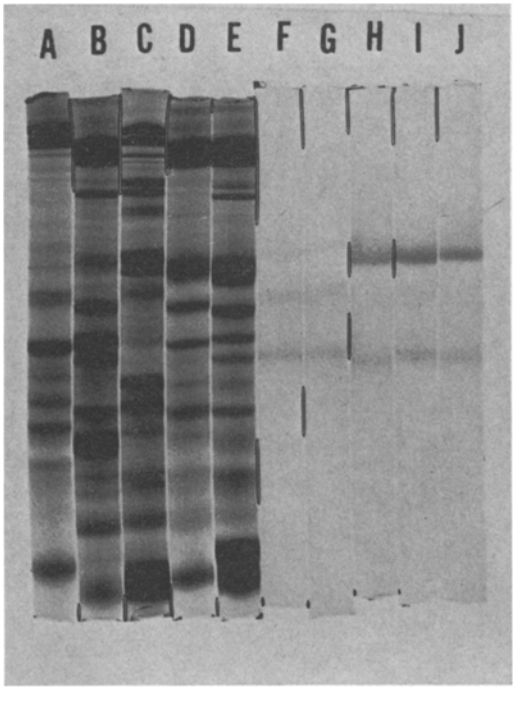

Fig. 2. Effects of α-chymotrypsin on animal red cells in SDS polyacrylamide disc gel electrophoresis. *A–E* Coomassie blue stain; *F–J* PAS stain; *A*, *F* human; *B*, *G* mouse; *C*, *H* sheep; *D*, *I* rabbit; *E*, *J* rat.

In the study of anion permeability, the efflux of $^{35}SO_4$ ions from previously labelled red blood cells was followed[2]. A medium composed of 150 mM NaCl, 4.8 mM Tris, pH 7.4, was used both for washing and incubation of red cell suspensions, while 20 mM of glucose was incubated in the final suspension. For labelling with $^{35}SO_4$, the cells were usually preincubated in the medium containing a trace of $H_2\,^{35}SO_4$. The cell density was 20 vol.-%. After 90 min at 37 °C, the cell suspensions were diluted 1:1 with the medium which additionally contained sufficient pronase to establish the desired final enzyme concentration. Incubation at 37 °C was continued for 60 min. At the end of second incubation period, the suspensions were diluted with ice cold $^{35}SO_4$-free medium containing no pronase and centrifuged. The cells were washed 2 more times with ice cold medium and finally resuspended in the same medium prewarmed to 37 °C. In the final suspension, the cell density was usually 5 vol.-%. After mixing, the samples were taken at suitable time intervals and centrifuged, and the radioactivity in the supernatant was followed. Determination of radioactivity and evaluation of data was performed as described by Poensgen and Passow[6].

Results and discussion. Figures 1 and 2 show the results of experiments. The gels which run in parallel, were stained for protein and for carbohydrate. The effect of pronase on red cell membrane was prominent in human and mouse cells (figure 1). The major band at mol.wt about 93,000 disappeared and simultaneously more protein was found in the mol.wt about 61,000. The gels L and N did not contain carbohydrate. The α-chymotrypsin attack was similar to that of pronase but the proteolysis was less vigorous (figure 2). The red cell membranes of rat, sheep and rabbit were not effected by these proteolytic enzymes. The effect of pronase on anion permeability was almost identical for human, mouse, sheep, rabbit and rat erythrocytes (figure 3).

5 G. Fairbanks, T. L. Steck and D. F. L. Wallach, Biochemistry *10*, 2606 (1971).

6 J. Poensgen and H. Passow, J. Membrane Biol. *6*, 210 (1971).

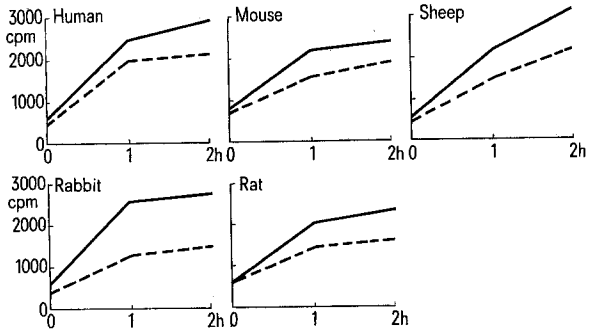

Fig. 3. Effects of pronase on $^{35}SO_4$ permeability on animal erythrocytes. ——, Control; ----, pronase 2 mg/ml.

The proteolytic enzymes are useful probes to determine which proteins of the membrane are exposed to the outside and potentially to determine the functional role of those proteins. The steric configuration of mouse glycoprotein may be similar to that of human glycoprotein. The glycoproteins of rat, sheep and rabbit erythrocytes are protective to these proteolytic enzymes. In human red blood cells, pronase reduces anion permeability, increases cation permeability and has no effect on the non-faccilitated component of the flux of the non-electrolyte. The protein is released much faster in the electrophoresis than the effects on anion permeability develope. There does not seem to be a causal relationship between the observed changes of the membranes' protein content and the permeability changes.

A mouse system for demonstrating the presence of inflammatory factors from human peripheral blood lymphocytes

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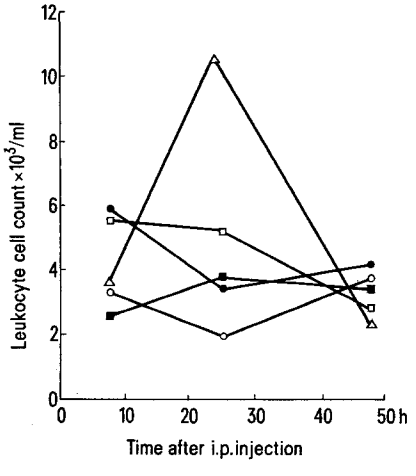
Summary. An assay system is described that allows the presence of inflammatory factors in supernatants from stimulated tuberculin-sensitive human peripheral blood lymphocytes to be demonstrated, by the induction of an inflammatory exudate in the peritoneal cavity of normal C57 BL mice.

The release of inflammation-inducing skin reactive factors (SRF) by antigen-stimulated sensitized animal and human lymphocytes has usually been demonstrated by the injection of active culture supernatants into the skin of normal unimmunized animals, e.g. guinea-pigs[2–6]. Although such a method allows the area of the inflammatory reaction induced to be assessed quantitatively, the type and number of cells infiltrating the test area can only be determined by microscopic examination of stained sections of the skin. We report here briefly a technique for the assay of inflammatory factors from human lymphocytes utilizing the ability of active supernatants to induce an exudative response in the peritoneal cavity of normal unimmunized mice.



Inflammatory exudates produced in mouse peritoneal cavities at various times after the injection i.p. of supernatants from sensitized lymphocyte cultures and control cultures. △—△, lymphocyte cultures incubated with PPD 0.5 μg/ml; ●—●, lymphocyte cultures incubated without antigen; ○—○, killed lymphocytes incubated with PPD 0.5 μg/ml; □—□, culture medium culture incubated with antigen; ■—■, culture medium culture incubated without antigen.

Materials and methods. Peripheral venous blood was obtained from healthy adults of either sex exhibiting delayed hypersensitivity skin reactions to tuberculin PPD. Peripheral blood lymphocytes were obtained by sedimenting the blood with a 6% solution of Dextran T110 (Pharmacia) in saline, 1 ml of dextran to 10 ml of blood. The leukocyte-rich supernatants were pipetted off and after washing were incubated at a cell concentration of 0.6×10^6 lymphocytes/ml in 2 ml of RPMI 1640 (GIBCO) containing penicillin 100 units/ml and streptomycin 100 μg/ml (GIBCO) with 20% heat inactivated autologous plasma. Cultures were stimulated with PPD (Connaught Laboratories) 0.5 μg/ml and control tubes either had an equal volume of saline added to the cells in culture instead of antigen, or PPD was added after the cells had been killed by heat at 60 °C for 30 min. Viability was checked by trypan blue exclusion. A further control consisted of culture medium alone or with antigen added but without cells. Supernatants were removed after 3 days incubation at 37 °C in sterile glass culture tubes in triplicate in 5% CO_2 and air. The supernatants were cleared of cells by centrifugation at 1000 × g for 10 min and were stored at −20 °C until used.

1 Section of Allergy and Clinical Immunology, Health Sciences Centre, 700 William Avenue, Winnipeg (Manitoba R3E OZ3, Canada).

2 B. Bloom and B. R. Bennett, Proc. nat. Acad. Sci. USA *59*, 756 (1968).
3 E. R. Heise and R. S. Weiser, J. Immun. *103*, 570 (1969).
4 D. C. Dumonde, R. A. Wolstencroft, G. S. Panayi, M. Matthew, J. Morley and W. T. Howson, Nature, Lond. *224*, 38 (1969).
5 E. Pick, T. Krejci, K. Cech and J. L. Turk, Immunology *17*, 741 (1969).
6 R. J. Warrington, S. K. Buehler and K. B. Roberts, Int. Archs. Allergy appl. Immun. *51*, 186 (1976).