Comparison of hemagglutination titer between Kaliss' method and the present 'plate' method

Antisera*	RBC	Method	Hemagglutination at $\log_2$ dilutions** 3	4	5	6	7	8	9	10	11
D-4	B10A	Kaliss	3	2	2	2	2	2	1	1	tr
		Plate	2	2	2	2	2	1	1	tr	—
D-5	B10A	Kaliss	1	1	2	2	2	1	1	1	tr
		Plate	1	2	2	2	2	2	1	tr	—
D-23	B10A	Kaliss	2	2	2	1	1	1	1	tr	—
		Plate	2	2	2	1	1	tr	tr	—	—

* Those alloantisera were given by the Transplantation Immunology Branch, NIAID, NIH. ** We adopted three grades of positive reaction, 3, 2 and 1. tr, trace; —, negative.

hematocrit value was 50.6 ± 3.5% for 12 specimens after 2700 × g for 1 min centrifugation in our case, a full capillary tube of blood (75 µl) contains about half the volume of RBC. We usually pour the full tube of blood into 10 ml Alsever solution immediately after bleeding, mix gently and wash 3 times with PBS by centrifugation at 540 × g for 3 min. Final volume was adjusted 5 ml to make 0.75% RBC suspension. By this procedure one can prepare RBC suspension at a given concentration very easily and fairly accurately. In the present study, we compared three concentrations of RBC suspension, 0.5%, 0.75% and 1.0%. A relatively clear result was obtained at 0.75%, though drastic effect of RBC concentration on the reliability of agglutination has not been observed within these ranges.

Titrations are performed in the Microtiter Plate for tissue culture (Cooke Laboratory Products, Virginia) made of rigid polystyrene, 12.7 × 9.5 cm and with 96 (8 × 12) flat-bottom wells. One drop (25 µl) of 1.2% or 0.6% PVP-BSA was dispensed to each well, then antisera were serially diluted by 25 µl loops. For this process, the microtitier plate should be kept somewhat declined to mix antiserum with diluent throughly. Finally 25 µl RBC suspension was dispensed to each well and shaken for about 10 sec by pressing down the microtitration plate on a voltex mixer. After incubation at 37 °C for 1 h and at 5 °C overnight, each plate was again shaken for 10 sec just before reading. As the specific hemagglutination seems to be fairly tight, one can read it repeatedly following preceding shaking. Without this, false positive agglutination is observed quite often even in the control. Microscopic observation of the hemagglutinations was conducted by the inverted type microscope for tissue culture equipped with X10 objective lens. The distance between the condenser lens and stage should be adjusted to give an appropriate contrast. The figure demonstrates the typical features of positive, trace and negative hemagglutination. Positive reaction is clearly discriminated from either trace or negative.

The sensitivity of the present method was compared with that of Kaliss' method using D-4, D-5 and D-23 alloantisera given by the Transplantation Immunology Branch, NIAID, NIH and B10A mouse RBC (table). Controls consisting of normal B10 mouse serum and B10A RBC have all shown negative reactions. Though the plate method seems to be slightly less sensitive than the original Kaliss' method, its operational simplicity could efficiently compensate the small defect.

Evaluation of humoral response with ragweed antigen in pregnant rats

H. M. Vijay and H. S. Buttar

Drug Research Laboratories, Health Protection Branch, Health and Welfare Canada, Ottawa (Ontario, Canada),
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Summary. No difference was observed between the homocytotropic (IgE) antibody titers in the sera of pregnant and non-pregnant rats immunized with DWSR, suggesting that there is no evidence of immunosuppressive factor in humoral response during pregnancy.

Recently, it has been reported that the large increases in the blood level of pregnancy-associated α-microglobulin or the whole pregnancy plasma have an inhibitory effect on several in vitro methods of immunological assessment[1]. This suppressive activity of plasma from pregnant women seemed to be levelled primarily at the cell-mediated immune response. Petrucco and his colleagues[2] reported that the cell-mediated response was significantly reduced in the second and third trimesters of pregnancy. Since, cellular hypersensitivity reactions are believed to be T-cell-mediated[3,4] these authors[2] gave an interpretation that T-cell activity is suppressed during pregnancy and this depression could be the result of immunosuppressive factors present in the lymphocytic environment.

Very little is known about the effects of pregnancy on humoral, i.e., immediate hypersensitivity reactions. The present study was designed to evaluate the humoral response with ragweed antigen in pregnant rats.

Experimental. Short ragweed 1:20 (Hollister-Stier, Mississauga, Ontario) was dialyzed against water at 4 °C and the nondialyzable part followed by lyophilization was used as an immunizing antigen, herein after designated as DWSR.

Nulliparous female outbred Wistar rats (225–250 g) (Woodlyn Farms, Guelph, Ontario), having free access to food and water, were used in this study. 2 females were paired overnight with one male proved fertile in breeding cages. Next morning, copulation was ascertained by finding sperms in vaginal smears or vaginal plug, and this was counted as day one pregnancy. On day 9 of pregnancy, the rats were randomly divided into 2 groups (10 animals per group). One group was immunized with a single i.p. injection of 1.0 ml of mixture containing

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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.

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

T. Kitao, K. Hattori and M. Takeshita

Department of Internal Medicine and Biochemistry, Kanazawa University School of Medicine, Kanazawa 920 (Japan), 12 October 1976

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%

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