Effect of nonspecific gamma globulin on passive sensitization in vitro at different temperatures


Specifically purified rabbit anti-dinitrophenyl antibodies were used to sensitize perfused, chopped guinea pig lung at 4°C and 37°C. Addition of nonspecific rabbit gamma globulin to purified rabbit antibody interfered with the rate of sensitization at 4°C much more than at 37°C. The data suggest that the temperature dependence of the sensitization process may largely reflect energy required to overcome interference by nonspecific gamma globulin.

The rate of passive sensitization in vitro of isolated guinea pig uterus,1 ileum,2 or sliced lung,3 by whole rabbit antiserum has been observed to be markedly dependent upon temperature. Recently, Feigen, Nielsen, and Terres4 studied the effect of temperature and antibody concentration on the physical adsorption of antibody to tissue and on quantity of histamine released for a given amount of adsorbed antibody. The amount of antibody physically adsorbed to ileal tissue was influenced by its bulk phase concentration but not by temperature. In contrast, antigen-induced histamine release at a given amount of antibody adsorbed was markedly dependent upon temperature, increasing as the temperature of the adsorption step was elevated. An important variable not considered in these previous studies is the presence of nonspecific gamma glob-
ulin in the antisera. Nonspecific gamma globulin may interfere with sensitization by competing with antibody for specific cell sites or by interacting directly with antibody so as to alter its immunologic behavior.

In the present experiments specifically purified rabbit antihapten antibodies were used to sensitize perfused, sliced, washed guinea pig lung. Addition of nonspecific rabbit gamma globulin to the purified antibody had a considerably greater effect in reducing the rate of sensitization at 4°C than at 37°C. The data reported herein suggest that the temperature dependence of the sensitization process may largely reflect energy required to overcome interference by nonspecific gamma globulin.

MATERIALS AND METHODS

Antigens

The hapten-conjugates, dinitrophenyl-bovine gamma globulin (DNP-BGG) and dinitrophenyl-bovine serum albumin (DNP-BSA), were prepared as previously described. The conjugates used contained a minimum of 30 haptenic groups per mole of protein.

Antibodies

Rabbits were immunized with DSP-EGG emulsified in Freund’s complete adjuvant, and anti-DNP antibodies were isolated according to the following procedure. One milligram of ethylenediaminetetraacetate (EDTA) was added to each milliliter of rabbit antiserum in order to prevent the addition of complement to the antigen-antibody precipitate; specific precipitation was obtained with DNP-fibrinogen. The precipitate was recovered, washed, and dissociated with DNP-ε-L-lysine. The DNP-fibrinogen was aggregated with streptomycin and separated by centrifugation; excess hapten was removed by prolonged dialysis. The protein concentration of the supernatant antibody solution was determined by the micro-Kjeldahl technique, and aliquots were stored at -70°C prior to use. Immunoelectrophoretic analysis of the purified rabbit antibody with sheep antiserum against rabbit gamma globulins yielded a single precipitin line in the gamma globulin region.

Nonspecific rabbit gamma globulin solutions

Normal rabbit gamma globulin, fraction II, was obtained from Mann Research Laboratories. Immunoelectrophoretic analysis with sheep antiserum against rabbit gamma globulins yielded a major precipitin arc in the gamma globulin region identical in appearance with that obtained with purified antibody. A stock solution of nonspecific rabbit gamma globulin, approximately 1.0 mg per milliliter, was prepared and the exact protein concentration was determined by the micro-Kjeldahl technique. Aliquots were stored at -70°C.

Preparation and sensitization of sliced lung tissue

Perfused, sliced, washed guinea pig lung tissue was prepared, as described previously, and sensitized in the following manner. Replicate tissue samples of approximately 100 mg, wet weight were suspended in 2 ml. Tyrode’s solu
tion and preincubated for 10 minutes at the temperature which was subsequently maintained during the sensitization period. The fluid phase was removed, the tissues were resuspended in 0.3 ml. Tyrode's solution and incubated at the appropriate temperature for 15 minutes prior to the addition of 0.3 ml. of Tyrode's solution containing antibody. The tissue samples, in a final volume of 0.6 ml., were gently agitated throughout the period of passive sensitization. Thereafter, the suspending medium was removed and all samples were washed three times in 2.0 ml. Tyrode's solution, without changing the temperature from that used during the sensitization period. The lung slices were then resuspended in 2.0 ml. Tyrode's solution, preincubated for 15 minutes at 37°C in a rocking bath, and challenged with 1 ml. of antigen solution. After 15 minutes' incubation at 37°C the suspending medium was separated from the tissue. This diffusate was either frozen immediately at 

\[-70^\circ\text{C.}\] or assayed for histamine by the bioassay procedure. Residual tissue histamine was extracted by boiling. The quantity of histamine released by antigen was expressed either in micrograms per gram wet lung tissue or as the per cent of total tissue histamine released. The values reported represent the average release from three replicate samples and have been corrected for spontaneous release from control samples; spontaneous release was consistently less than 0.05 \(\mu g\) per gram wet lung tissue or 0.5 per cent of the total tissue histamine.

**RESULTS**

**Standard conditions for sensitization at 37°C.**

In several experiments it was found that 0.45 to 4.5 \(\mu g\) rabbit anti-DNP antibody protein sensitized 100 mg. guinea pig lung samples for maximal histamine release on exposure to antigen, DNP-BSA, 50 \(\mu g\) protein per milliliter. This concentration of antigen was used routinely after it was found to yield maximal histamine release from tissue sensitized with quantities of antibody varying from 0.015 \(\mu g\) to 45 \(\mu g\) antibody protein. Tissue was exposed to antibody for 21/2 hours at 37°C; extending the period of sensitization at 37°C beyond 21/2 hours did not increase histamine release upon subsequent exposure to antigen.

**Effect of temperature on sensitization by varying concentrations of antibody**

The results of 13 experiments in which replicate samples of lung tissue were sensitized for 21/2 hours at either 37°C or 4°C are depicted in Fig. 1. Intermediate temperatures were investigated in some experiments, and in two others the comparison was limited to 37°C and 18°C. The quantity of antibody used for sensitization varied from 0.2 to 45 \(\mu g\) protein, thereby including the range 0.45 to 4.5 \(\mu g\) previously found to yield maximal histamine release at 37°C. There was no consistent difference in the sensitization achieved with 4.5, 6.0, and 45 \(\mu g\) antibody protein during incubation for 21/2 hours at either 4°C or 37°C. In three experiments performed with smaller amounts of antibody, 0.2 \(\mu g\), the data suggested that sensitization achieved at 4°C was somewhat less than that attained at 37°C.
Effect of temperature on sensitization of chopped guinea pig lung by various concentrations of purified rabbit anti-hapten antibody. In each of 13 separate experiments, tissue was sensitized at 4°C and 37°C, respectively for 2½ hours with a given concentration of antibody. Sensitized tissue was preincubated at 37°C and challenged with DNP-BSA, 50 µg per milliliter.

Effect of temperature on sensitization by different concentrations of antibody. Replicate samples of the same lung tissue were sensitized at 4°C and 37°C, respectively with varying concentrations of antibody.

The effect of temperature on sensitization of lung tissue with small amounts of antibody was further explored (Fig. 2). An equal amount of histamine was released following addition of antigen to tissue sensitized at 37°C during 2½ hours with either 6.0, or 0.6 µg antibody protein. At 4°C, sensitization with 6.0 µg antibody was equivalent to that obtained at 37°C, but only two-thirds as much histamine was released from tissue sensitized at this temperature with 0.6 µg of antibody protein. Using 0.2 µg antibody protein, approximately one-fourth as much histamine was released from tissue incubated with antibody at 4°C as from tissue incubated at 37°C.

The effect of incubation for ½, 1½, and 2½ hours at 4°C and 37°C on sensitization was studied with 3.0 and 6.0 µg of purified antibody. There was...
Effect of temperature on sensitization

There was no significant difference in the rate of sensitization measured at these times. Extending the incubation period beyond 2½ hours at 37° C. actually decreased sensitization, possibly due to deterioration of the tissue. In contrast (Fig. 3), extending the sensitization period at 4° C. allowed progressively greater sensitization; the maximum histamine release from tissue sensitized at 4° with 0.1 μg to 3.0 μg antibody protein exceeded that observed at 37° C. Spontaneous histamine release from control tissue samples was not increased after 18 hours at 4° C.

The effect of varying concentrations of nonspecific gamma globulin on sensitization at 4° C. and 37° C., respectively

Various mixtures of nonspecific rabbit gamma globulin and purified rabbit antibody were used to sensitize guinea pig lung tissue at 4° C. and 37° C., respectively. In the two experiments shown in Fig. 4, the antibody concentrations used, 4.5 or 6.0 μg produced identical sensitization at the two temperatures. The presence of 30 μg of a nonspecific gamma globulin, giving mixtures containing 14 per cent and 17 per cent specific antibody, respectively, interfered with sensitization at 4° C. considerably more than at 37° C. Sensitization in 2½ hours, at either temperature, was prevented by the presence of 150 μg of nonspecific gamma globulin. The experiment depicted in Fig. 5 presents similar results: 3 μg of rabbit antibody gave equal sensitization during 2½ hours at 4° C. and 37° C., respectively; in the presence of 15 μg of nonspecific gamma globulin, sensitization at 4° C. fell to one-half that achieved at 37° C.; in the presence of 30 μg of nonspecific gamma globulin sensitization at 4° C. was reduced to one-fourth that obtained at 37° C.

Effect of varying time of sensitization at 4° C. and 37° C. in the presence of nonspecific gamma globulin

Additional evidence that the principal action of nonspecific gamma globulin was to reduce the rate of sensitization at 4° C., compared to that at 37° C., is shown in Fig. 6. In contrast with results obtained at 37° C., only minimal sensitization of chopped guinea pig lung was achieved with a mixture of 3.0 μg of antibody protein and 15 μg of nonspecific gamma globulin during a 2½ hour incubation at 4° C. By extending the incubation period at 4° C. to 18 hours, the final sensitization achieved approximated that obtained in 2½ hours at 37° C.

DISCUSSION

Current concepts of sensitization focus upon the ability of antibodies to interact with target cells or limited sites on such cells, so that subsequent union with antigen leads to histamine release. Since it is likely that a limited number of antibody molecules are required to sensitize a single target cell, histamine release directly reflects the quantity of antibody applied to tissue over only a limited range of antibody concentrations; outside of this range a direct relationship may no longer obtain. Recent studies indicate that most animal species investigated, including man, guinea pig, dog, and rat, and
Fig. 3
Effect of duration of incubation at 4°C and 37°C on sensitization. Replicate samples of the same lung tissue were sensitized at 4°C and 37°C, respectively with 0.1 μg rabbit anti-DNP for varying periods of time.

Fig. 4
Effect of various amounts of nonspecific rabbit gamma globulin on sensitization at 4°C and 37°C. Specific antibody, rabbit anti-DNP, and nonspecific rabbit gamma globulin were mixed together for 10 minutes at room temperature prior to incubation with the tissue samples.

Fig. 5
Effect of various amounts of nonspecific rabbit gamma globulin on sensitization at 4°C and 37°C. Three micrograms of rabbit anti-DNP and varying amounts of nonspecific rabbit gamma globulin were mixed together for 10 minutes at room temperature prior to incubation with the tissue samples.
The increased sensitization achieved with small amounts of antibody at 37°C compared to 4°C might be attributable to an increased rate of uptake of antibody by tissue at the higher temperature. Alternatively increased temperature may favor molecular rearrangement on the tissue, thus bringing more antibody molecules into the required spatial arrangement on target cells, or temperature may enhance penetration of antibody to target cells within the
blocks of lung tissue. The first possibility is unlikely, in view of the findings of Feigen and associates\(^4\) that the amount of antibody physically adsorbed to guinea pig ileum in vitro was influenced only by the concentration of antibody in the incubation medium and was not influenced by the temperature maintained during incubation. Their additional finding that sensitization, as reflected by the quantity of histamine released for a given extent of physical adsorption of antibody, was greatly increased as the temperature of the adsorption step was elevated, might be explained by either of the other alternatives proposed above.

In the present experiments, as the concentration of purified antibody used for sensitization was increased, a point was reached at which equivalent amounts of histamine were released from tissue incubated with antibody for 2\(\frac{1}{2}\) hours at either 4\(^\circ\) C. or 37\(^\circ\) C. (Figs. 1, 2). At such concentrations of antibody, adsorption alone seemed to assure that sufficient antibody molecules reached target cells. Under these circumstances the inability to demonstrate a temperature effect might be attributed to a complete saturation of the sites on the target cells. However, experiments in which incubation of tissue with antibody at 4\(^\circ\) C. was prolonged to 18 hours indicated that it was possible to exceed significantly the sensitization achieved in 2\(\frac{1}{2}\) hours at 37\(^\circ\) C. These results make it unlikely that saturation of sites limited sensitization at 37\(^\circ\) C. Brocklehurst and co-workers\(^5\) have demonstrated that chopped lung tissue deteriorates significantly after 2 to 3 hours' incubation at 37\(^\circ\) C. The process of deterioration may begin even earlier, and this may limit the ability to demonstrate a temperature effect on sensitization of tissue with higher concentrations of antibody.

Experiments performed with artificial mixtures of purified anti-hapten antibody and nonspecific gamma globulin indicated that the passive sensitization achieved with such mixtures at either 37\(^\circ\) C. or 4\(^\circ\) C. markedly diminished, as the concentration of nonspecific gamma globulin was increased. The addition of nonspecific gamma globulin to purified rabbit anti-DNP interfered with sensitization at 4\(^\circ\) C. much more than with sensitization at 37\(^\circ\) C. (Figs. 4 to 6). Prolonging the period of incubation of tissue with artificial mixtures at 4\(^\circ\) C. to 18 hours resulted in a final sensitization similar to that achieved at 37\(^\circ\) C. in 2\(\frac{1}{2}\) hours' incubation (Fig. 6). The data suggest that nonspecific gamma globulin affects primarily the rate of sensitization rather than the ability of the tissue to become sensitized.

It is unlikely that temperature aids in overcoming the effect of nonspecific gamma globulin on passive sensitization by promoting increased penetration of lung tissue. The antibody solution reaching remote target cells would still have the same unfavorable proportion of specific to nonspecific gamma globulin molecules at either temperature. Nonspecific gamma globulin residing on tissue, or adsorbed simultaneously with antibody, probably increases the need for molecular rearrangement in order to achieve the appropriate antibody relationship on the target cell. This rearrangement can be accelerated by increasing the temperature or prolonging the duration of incubation. The dependence of sensitization upon temperature, which led others to calculate
a considerable energy of activation, \(^a\) may largely reflect energy required to overcome interference by nonspecific gamma globulin.

REFERENCES