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Enhancement of Reagin Formation in Rabbits by Passively Administered 19S Antibody

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Summary. Rabbits immunized with haemocyanin in Freund's complete adjuvant responded in almost all cases with the formation of serum homocytotropic (reaginic) antibodies. Intravenous administration of 19S antibodies 1 day before or after antigenic stimulation resulted in an enhancement of the formation of the homocytotropic antibodies. No significant effect on the synthesis of 7S and 19S antibodies, reactive in passive haemagglutination, was observed. The results are discussed in relation to hyposensitization therapy.

INTRODUCTION

The formation of 19S and 7S antibodies may be suppressed by passive administration of 7S antibody (reviewed by Uhr and Möller, 1968). Recently it has been shown that passively administered 7S antibody may inhibit the primary formation of homocytotropic (reaginic) antibodies as well (Strannegård and Belin, 1970). It has been observed, however, with certain antigens, that relatively small amounts of passive antibody may actually enhance the antibody response (Terres and Wolins, 1961; Segre and Kaeberle, 1962; Terres and Stoner, 1962; Clarke, Donohoe, McConnell, Woodrow, Finn, Krevans, Kulke, Lehane and Sheppard, 1963; Lodee, Segre and Mayers, 1964; Möller and Wigzell, 1965). The explanation for this seemingly paradoxical stimulating effect of passive antibody is not clear, but it has been suggested that cytophilically attached antibody molecules may capture antigenic particles by combining with some antigenic determinants, thereby offering other antigenic determinants, that have remained free, to the target lymphocytes (Henry and Jerne, 1968). Recently Henry and Jerne (1968, 1969) have provided evidence that 19S but not 7S passive antibody is the enhancing factor and they have been able to predict the magnitude of an immune response on the basis of the proportions of 7S and 19S antibody in a given mixture of passively administered antibody.

The aim of the present investigation was to study the effect of passive 19S antibody on reagin synthesis.

MATERIALS AND METHODS

Antigen. Haemocyanin was collected from the haemolymph of *Buccinum undatum* and prepared as previously described (Strannegård and Belin, 1970).

The techniques for passive haemagglutination (HA), homologous passive cutaneous anaphylaxis (PCA), treatment with 2-mercaptoethanol (2-ME) and density gradient ultracentrifugation have been described (Strannegård and Belin, 1970).

Preparation of 19S antibodies. 19S antibodies were prepared according to Henry and Jerne (1968) by euglobulin precipitation of pools of sera from rabbits immunized 2 or 3 weeks prior to the bleeding with 50 μg of haemocyanin. The euglobulin preparations contained 2-mercaptoethanol sensitive anti-haemocyanin antibodies as measured in the passive haemagglutination (HA) assay (Stavitsky, 1964). In the following, the antibodies in the preparations are referred to as 19S antibodies. Immunoelectrophoretic analysis using goat and guinea-pig anti-rabbit globulin sera showed that the preparations contained IgM but no detectable IgG. The preparations gave negative reactions when tested in the homologous PCA test against haemocyanin.

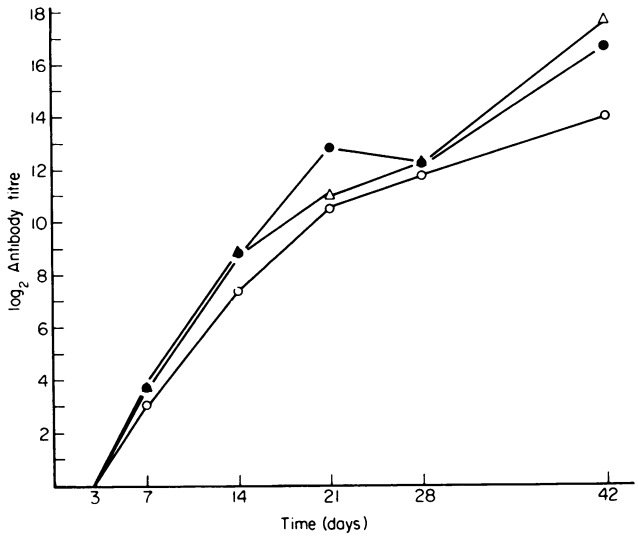

FIG. 1. Antibody formation after passive administration of 19S antibody 1 day before or after antigenic stimulation. ●, No passive antibody; ○, passive antibody day −1; △, passive antibody day +1. Each point represents the geometric mean titre from eight rabbits, as measured in the passive haemagglutination test.

RESULTS

EFFECT OF PASSIVE 19S ANTIBODY ON THE FORMATION OF 7S AND 19S HAEMAGGLUTINATING ANTIBODIES

Twenty-four albino rabbits weighing 2·0–2·5 kg were immunized with 50 μg haemocyanin in Freund's complete adjuvant in the hind foot pads. This antigenic stimulus regularly evoked a marked antibody response in the rabbits. Analysis of the antibodies in sera obtained at various time intervals with 2-ME treatment and sucrose density gradient ultracentrifugation revealed a 7S and 19S antibody response, similar to that described previously (Strannegård and Belin, 1970). The 19S antibodies persisted for at least 6 weeks following the antigenic stimulation. Intravenous administration of a 19S antibody preparation, either 1 day before or 1 day after the antigenic stimulation, did not significantly influence the pattern of response or the serum titres of the HA antibodies (Fig. 1). The experiments were performed with two 19S antibody preparations, one with a titre in the HA test of 1/4096 and one with a titre of 1/1024, and each rabbit received 5 ml of either

preparation. Neither of the preparations influenced the response of 7S and 19S antibodies significantly.

EFFECT OF PASSIVE 19S ANTIBODY ON REAGIN FORMATION

Twenty-three out of the twenty-four rabbits injected with 50 μg haemocyanin responded with formation of reaginic antibodies as measured in the homologous passive cutaneous anaphylaxis test. The kinetics of reagin formation followed the same pattern as described previously (Strannegård and Belin, 1970), although the synthesis of reaginic antibodies appeared to be somewhat delayed compared with the earlier results. Passive administration of either of the two 19S antibody preparations described above stimulated reagin synthesis. The difference in magnitude of reagin response obtained with 19S antibody administered on day +1 and −1 was evident at a significance level of 0·05. The reagin synthesis was

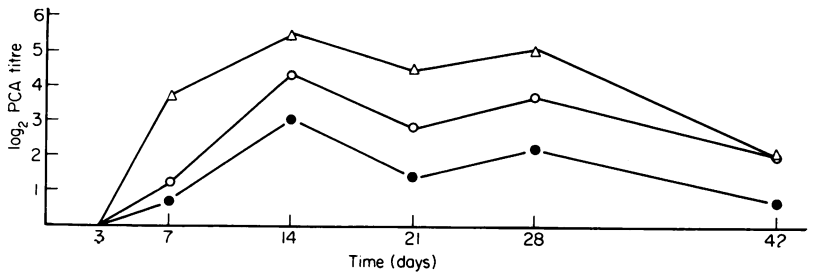

FIG. 2. Reagin formation after passive administration of 19S antibody. ●, No passive antibody; ○, passive antibody day −1; △, passive antibody day +1. Each point represents the geometric mean reagin titre from eight rabbits as measured in the homologous passive cutaneous anaphylaxis (PCA) assay. Statistical analysis (Wilcoxon test) reveals that the 19S antibody preparations significantly enhance the reagin response whether given 1 day before, or 1 day after, the antigen (significance levels $<0{\cdot}05$ and $<0{\cdot}01$, respectively).

thus more enhanced when 19S antibody was administered 1 day after, rather than 1 day before antigen injection (Fig. 2). Statistical analysis (Wilcoxon test) on the pooled data obtained from experiments performed on the twenty-four rabbits showed that 19S antibody administered 1 day after or 1 day before antigenic stimulation significantly enhanced the reagin response. The probability of equal magnitude of reagin response was rejected on the basis of significance levels of 0·01 and 0·05, respectively.

DISCUSSION

The present experiments show that passively administered 19S antibody preparations may enhance reagin formation in rabbits. Several lines of evidence indicate that 7S antibody suppresses reagin synthesis (Strannegård and Yurchision, 1969a, b; Strannegård and Belin, 1970), and it thus seems probable that reagin formation is subject to both positive and negative antibody-induced regulatory feed-back mechanisms. With the 19S antibody preparations used, no significant effect on the formation of 7S and 19S antibodies was observed. These results are in contrast to those of Henry and Jerne (1968, 1969), but may be explained by the fact that relatively low concentrations of 19S antibody were used in the present experiments. It is also possible that the discrepancies observed

reflect true differences in the systems. It should be noted that the feed-back inhibition of 19S antibody synthesis by 7S antibody which is very evident in the sheep red blood cell system (Möller and Wigzell, 1965) does not operate efficiently in the key-hole limpet haemocyanin system (Dixon, Jacot-Guillarmod and McConahey, 1967).

Since the isolation method used by us should result in a contamination of about 5 per cent 7S antibody in the 19S preparation (Henry and Jerne, 1969) our results do not exclude the possibility that 7S antibody may have an enhancing effect on reagin synthesis, when administered in low concentrations. This would not appear to be very likely, however, since it has not been convincingly demonstrated in any system that 7S antibody has any stimulating effect on antibody response.

Macroglobulin antibody had a more marked enhancing effect on reagin synthesis when administered 1 day after than when given 1 day before the antigen. It is possible that the 19S antibodies which have a rather short half-life might have occurred in too low concentrations to have any effect on reagin synthesis if administered prior to antigen injection. However, Henry and Jerne (1969), in their experimental system, observed a more marked enhancing effect of 19S antibody which had been given 24 hours before the antigen rather than 1 hour before.

Hyposensitization therapy of allergic disorders is often performed with low doses of antigen. A low dose of antigen may elicit 19S but not 7S antibody formation (Uhr and Finkelstein, 1963; Svehag and Mandel, 1964). It is possible that with repeated injections of small amounts of antigen, previously formed 19S antibody may enhance rather than suppress reagin formation. The present experiments describe the influence of 19S antibody on the primary reagin response, but a situation similar to that encountered in hyposensitization therapy has not been studied experimentally as yet. On the available experimental basis it seems logical to assume, however, that hyposensitization should be performed with antigen doses that give rise to 7S and not only 19S antibody formation. This assumption gets support from the results of clinical investigations which show a better effect of hyposensitization with high instead of low antigen doses (Johnstone and Dutton, 1968).

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REFERENCES

Clarke, C. A., Donohoe, W. T. A., McConnell, R. B., Woodrow, J. C., Finn, R., Krevans, J. R., Kulke, W., Lehane, D. and Sheppard, P. M. (1963). 'Further experimental studies on the prevention of Rh haemolytic disease.' *Brit. med. J.*, i, 979.

Dixon, F. J., Jacot-Guillarmod, H. and McConahey, P. (1967). 'The effect of passively administered antibody on antibody synthesis.' *J. exp. Med.*, **125**, 1119.

Henry, C. and Jerne, N. K. (1968). 'Competition of 19S and 7S antigen receptors in the regulation of the primary immune response.' *J. exp. Med.*, **133**, 128.

Henry, C. and Jerne, N. K. (1969). 'The depressive effect of 7S antibody and the enhancing effect of 19S antibody in the regulation of the primary immune response.' *Nobel Symposium*, No. 3, p. 421. Almqvist & Wiksell, Stockholm.

Johnstone, D. E. and Dutton, A. (1968). 'The value of hyposensitization therapy for bronchial asthma in children—A 14 year study.' *Pediatrics*, **42**, 793.

Lodee, R. F., Segre, D. and Mayers, W. L. (1964). 'The immunologic behavior of baby pigs. IV. Intestinal absorption and persistence of 6.6S and 19S antibodies of ovine origin and their role in the immunologic competence of baby pigs.' *J. Immunol.*, **93**, 576.

Möller, G. and Wigzell, H. (1965). 'Antibody synthesis at the cellular level. Antibody-induced

suppression of 19S and 7S antibody response.' *J. exp. Med.*, **121**, 969.

SEGRE, D. and KAEBERLE, M. L. (1962). 'The immunologic behavior of baby pigs. I. Production of antibodies in three-week-old pigs.' *J. Immunol.*, **89**, 782.

STAVITSKY, A. B. (1954). 'Micromethods for study of proteins and antibodies. Procedure and general applications of haemagglutination inhibition reactions with tannic acid and protein-treated red blood cells.' *J. Immunol.*, **72**, 360.

STRANNEGÅRD, Ö. and BELIN, L. (1970). 'Suppression of reagin synthesis in rabbits by passively administered antibody.' *Immunology*, **18**, 773.

STRANNEGÅRD, Ö. and YURCHISION, A. (1969a). 'Formation of rabbit reaginic antibodies to protein and hapten-protein conjugates.' *Immunology*, **16**, 387.

STRANNEGÅRD, Ö. and YURCHISION, A. (1969b). 'Formation of agglutinating and reaginic antibodies in rabbits following oral administration of soluble and particulate antigens.' *Int. Arch. Allergy*, **35**, 579.

SVEHAG, S. E. and MANDEL, M. (1964). 'The formation and properties of poliovirus neutralizing antibody. II. 19S and 7S antibody formation: differences in antigen dose requirement for sustained synthesis, anamnesis and sensitivity to X-irradiation.' *J. exp. Med.*, **119**, 21.

TERRES, G. and STONER, R. D. (1962). 'Specificity of enhanced immunological sensitization of mice following injections of antigens and specific antisera.' *Proc. Soc. exp. Biol. (N.Y.)*, **109**, 88.

TERRES, G. and WOLINS, W. (1961). 'Enhanced immunological sensitization of mice by the simultaneous injection of antigen and specific antiserum. I. Effect of varying the amount of antigen used relative to the antiserum.' *J. Immunol.*, **86**, 361.

UHR, J. W. and FINKELSTEIN, M. S. (1963). 'Antibody formation. IV. Formation of rapidly and slowly sedimenting antibodies and immunological memory to bacteriophage ΦX174.' *J. exp. Med.*, **117**, 457.

UHR, J. W. and MÖLLER, G. (1968). 'Regulatory effect of antibody on the immune response.' *Advanc. Immunol.*, **8**, 81.