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Antigen in Tissues

V. EFFECT OF ENDOTOXIN ON THE FATE OF, AND ON THE IMMUNE RESPONSE TO, SERUM ALBUMIN AND TO ALBUMIN–ANTIBODY COMPLEXES*

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Summary. Bacterial endotoxin was injected into rat hind footpads together with bacterial flagellin and ^{125}I-labelled human serum albumin (HSA); the latter was used unmodified or heat denatured (H.HSA) or as an HSA–antibody complex. Endotoxin did not affect the trapping, retention nor localization of the labelled HSA in the popliteal and aortic lymph nodes, whether the antigen had been injected as HSA, H.HSA or as an HSA–antibody complex.

If endotoxin was injected at the same time as: (1) flagellin, there was an increased production of anti-flagellin antibody; and (2) H.HSA or HSA–antibody complex, detectable amounts of anti-HSA antibody were produced. When H.HSA and endotoxin were injected, the primary response was long lived yet the period of induction of antibody formation and of antigen persistence in the lymphoid tissues was short. If, during the primary antibody response to H.HSA, the animals were challenged with HSA, equally strong secondary antibody responses occurred with an HSA–antibody complex or with HSA alone.

The results were interpreted in terms of the tissue localization pattern of H.HSA (medullary macrophage) and HSA–antibody complex (medulla and lymphoid follicles). It was suggested that: (1) induction of antibody formation and priming of cells for a secondary antibody response might occur following localization of the antigen in the medulla and that antigen localization in the lymphoid follicles might not be a strict requirement for this; and (2) the follicular localization of antigen might be the preferential mechanism for the firing of a secondary antibody response.

INTRODUCTION

An antigen, after injection into animals, drains away from the injection site and some is recovered in lymphoid tissues. Some antigens, such as bacterial flagella became localized in lymphoid organs predominantly in one or two areas, in macrophages in the medulla (lymph nodes) or red pulp (spleen) and in the lymphoid follicles (node) or white pulp (spleen). Antigens may persist or be rapidly degraded in macrophages but frequently are retained for long periods of time in the lymphoid follicles or white pulp where the antigen, probably as an antigen–antibody complex, is largely present on the surface of reticular cells or histiocytes (Mitchell and Abbot, 1965; Balfour and Humphrey, 1966). Other

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antigens such as human serum albumin (HSA) become widely spread throughout lymphoid tissues but are poorly retained in them. In earlier work, conditions were described whereby HSA was modified so that the extent of trapping and retention in the lymph nodes draining the injection site was increased. In one case, heat treatment of HSA was found to result in increased trapping of the antigen and the trapped antigen if labelled with radioisotope was found to be localized almost exclusively in the medullary macrophages (Lang and Ada, 1967a). On the other hand increased trapping of HSA by the lymph node also occurred if the labelled antigen was premixed with specific antibody before injection but in this case the label was found both in medullary macrophages and over cells in the lymphoid follicles. The labelled antigen rapidly disappeared from medullary macrophages but persisted in the follicles (Lang and Ada, 1967b). Despite the increased retention of HSA in lymph nodes in both of these situations there was no detectable formation of antibody using a reasonably sensitive method of titration. HSA, untreated or pretreated as described above, was therefore injected together with adjuvant (bacterial endotoxin) and the effect of this treatment on antigen retention, on antigen localization and on antibody formation was determined. The results are reported in this paper.

MATERIALS AND METHODS

Antigens

Flagellin was prepared from flagella (*S. adelaide*) as described previously (Ada, Nossal, Pye and Abbot, 1964a). Purified HSA was a product of Behringwerke, Marburg-Lahn. For some experiments dilute preparations (0·01 per cent w/v) of HSA in phosphate buffered saline (pH 7·3) were heated at 90° for 15 minutes before injection (Lang and Ada, 1967a).

Endotoxin

A preparation of lipopolysaccharide from *E. coli* BV. was used (Westphal, Luderitz and Bister, 1952). It was supplied by Dr C. Jenkins to whom our thanks are due. The opalescent endotoxin solution (800–1000 μg/ml in saline) was prepared immediately before use and 25 μl injected into each footpad.

Iodination of antigens

Antigens were iodinated with carrier-free preparations of ^{125}I (preparation IMS. 3 in neutral solution at a concentration of about 100 mc/ml), by the procedure described elsewhere (Ada, Nossal and Pye, 1964b). Radioactivity of organs was estimated in a well type scintillation counter (approximate efficiency 60 per cent) or, in the case of highly active tissues, in a scintillation counter with variable geometry. The specific activity of HSA was about 6 μc/μg. Usually labelled proteins were injected into rats within 2 hours after iodination.

Animals

Young rats (about 200 g weight) of the Wistar strain were bred by random mating and either sex was used. Six-month-old rabbits were used for the production of hyperimmune serum. All animals were fed *ad libitum* and after an injection of labelled antigen, the water given to animals contained 4·5 g NaCl and 0·1 g KI per litre (Humphrey and Turk, 1961).

Injection of antigens and treatment of tissues

Antigens, either alone in saline or mixed with normal or immune serum were injected into the hind footpad of rats (volume 50–200 μl), containing usually 10 μg of HSA or 1 μg of flagellin. Endotoxin (40–50 μg in 10–50 μl/rat) was injected separately. In some experiments animals were bled at intervals for periods up to 10 weeks after the initial injection of antigen. Other animals were killed at times which varied from 1 hour to 2 weeks after antigen injection. The popliteal and aortic nodes were removed immediately, weighed, placed in 10 per cent formol saline and their radioactivity determined. The radioactivity of feet, spleen and sera were also determined.

Autoradiography

Tissues in 10 per cent formol saline were processed for autoradiography by serial passage through alcohol solutions and chloroform, embedded in wax, sections cut and floated onto gelatin coated slides. The slides were dipped into NTB_2 photographic emulsion (Kodak) and exposed for times determined by the amount of isotope present in the tissue after processing. These times varied from several hours to several weeks, but to allow comparisons to be made a 60-day exposure (one half-life of the isotope) was carried out routinely on one set of sections from each issue. Popliteal and aortic nodes were regularly examined. The level of radioactivity in spleens was too low for this purpose.

Production of hyperimmune sera

Rabbits, rats or mice were injected subcutaneously with antigen mixed with complete Freund's adjuvant and a second similar injection of antigen was given about 4 weeks later. The animals were bled between 1 and 2 weeks after the second injection of antigen. Sera were collected and kept frozen until required. The antibody activity in the rat anti-HSA sera was examined both by column chromatography on Sephadex G-200 and by the sedimentation properties when centrifuged in sucrose gradients. By both criteria, the antibody activity was found to be associated mainly with IgG.

Estimation of serum antibody levels

Antibody to unmodified HSA was estimated by either one of two techniques—by a procedure using tanned, formolized red cells sensitized with HSA or by the Farr (1958) procedure (Lang and Ada, 1967b). Examination of both low and high titre sera showed that the methods gave comparable answers. For example three sera were compared by each technique and gave the following titres: (1) by haemagglutination, A = 16,000, B = 160, C = 720; and (2) by Farr test (antigen binding capacity), A = 835 μg/ml, B = 8·2 μg/ml, C = 73·5 μg/ml. The sensitivity of the Farr method was about 0·3 μg/ml. Antibody to flagellin was estimated by the immobilization technique (Ada *et al.*, 1964a), which had a sensitivity of about 15 ng antibody/ml. When antigen–antibody complexes or precipitates were used, an amount of antibody calculated to bind completely with the antigen, was used. For example, 10 μg of HSA was mixed with 12·5 μl of serum having an antigen binding capacity of 800 μg/ml. In experiments where labelled antigen was used and precipitation allowed to happen before injection more than 90 per cent of the label was recovered in the precipitate.

Determination of antibody species

Some sera containing antibody were fractionated into three peaks of protein by passage through a column of Sephadex G-200 (Pharmacia) previously equilibrated with phosphate

buffered saline, pH 7·3. Calibration with standard solutions of IgM and IgG showed that antibody activity in the first protein peak was IgM while activity in the second peak was IgG.

RESULTS

It has previously been shown that several factors may play a role in influencing the adjuvant action of endotoxin in the antibody response to an antigen. One such factor is the time elapsing between the injection of antigen and endotoxin. Kind and Johnson (1959) and Luecke and Sibal (1962) injected antigen and endotoxin at different times intravenously into rabbits and chickens respectively. Both showed that endotoxin given simultaneously with the antigen resulted in the highest antibody titres. As different animals (rats) and a different route of injection were to be used in the present experiments, this aspect was re-investigated.

THE EFFECT ON ANTIBODY PRODUCTION OF THE VARIATION IN TIME BETWEEN INJECTION OF HEAT DENATURED HSA (H.HSA) AND OF ENDOTOXIN

Endotoxin (40 μg) was injected into groups of rats either 1 day previously, at the same time as, or at 1, 2, 3 or 7 days subsequent to the injection of antigen (10 μg H.HSA). The rats were bled at weekly intervals up to 28 days after the antigen injection. Antibody

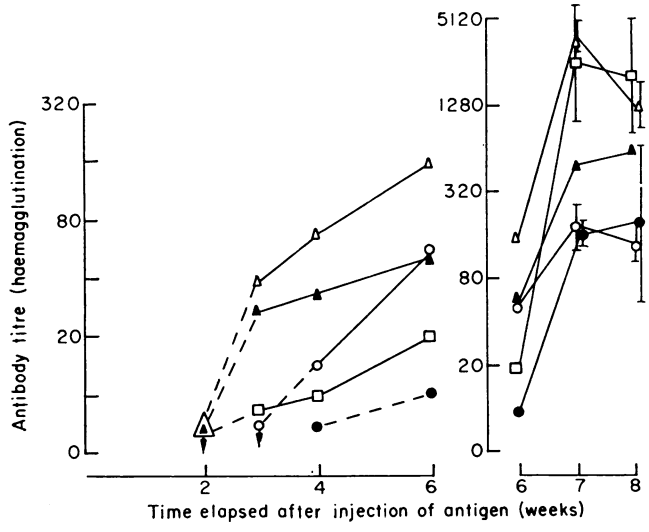

FIG. 1. The primary and secondary antibody response of rats injected initially with H.HSA (10 μg) and 6 weeks later with HSA (10 μg). Endotoxin (40 μg) was injected at various times before or after the injection of H.HSA. Time of injection of endotoxin relative to injection of H.HSA: Beforehand—8 hours (○); 4 hours (●); simultaneously (△); afterwards—4 hours (▲); 8 hours (□). Vertical lines represent the standard deviation of the mean values. One set of calculations is not given as in this group (▲), one rat responded very poorly so that the standard deviations were unduly high.

was detected only in the group of rats which had been injected simultaneously with antigen and with endotoxin (mean haemagglutination titre at 21 days = 240). In a second experiment, the doses of antigen and endotoxin injected were similar to those quoted above but the endotoxin was injected either 8 or 4 hours beforehand, at the same

time as, or 4 or 8 hours after the injection of antigen. The antibody titres obtained are shown in Fig. 1. The highest titres occurred in rats which had been injected simultaneously with H.HSA and endotoxin. Six weeks after the first injection of H.HSA, all rats were injected with 10 μg HSA. Those rats which had initially received endotoxin at the same time as or subsequent to the injection of H.HSA gave, after a later injection of HSA, antibody titres which were significantly higher than the titres from rats which had initially received endotoxin prior to the injection of H.HSA. In a separate experiment, it was found that endotoxin (40 μg) injected simultaneously into rats with HSA (10 μg) did not cause detectable antibody formation in a 6-week period after the injection.

These experiments showed that the maximum production of antibody to H.HSA occurred when endotoxin was injected either at the same time as or shortly after the injection of H.HSA into the hind footpads of rats. As seen below, the time at which H.HSA was present in greatest amount in the lymph nodes draining the injection site and in the spleen was also within a few hours after injection. It seemed possible that if high levels of antigen could be maintained in the draining lymph node for some days after the injection of antigen, then injection of endotoxin some days after H.HSA injection might still cause detectable anti-HSA antibody formation. It was found that the level of radioactivity in lymphoid organs 2 hours after injection (node 62 ng, spleen 24 ng), was about fifty times higher than the amount present in these organs 3 days later. Groups of rats were therefore injected with 1 mg of a labelled H.HSA preparation, i.e. 100 times more than in the former experiments. The retention of this antigen in tissues expressed as a percentage of the amount injected was similar to that when the smaller dose was injected. For example 3 days after the injection of 1 mg of antigen the radioactivity in lymph nodes and spleens was about 0·01 per cent of the amount injected, i.e. about 100 ng of antigen. Rats injected with 1 mg of H.HSA were injected either at the same time or 1, 2 or 3 days later with 40 μg endotoxin. Each group of rats now produced antibody. For example, rats which had received endotoxin three days after H.HSA had a mean serum antibody titre of 80 (haemagglutination) 3 weeks after antigen injection.

This experiment suggested that the effectiveness of endotoxin as an adjuvant might be related to the level of antigen present in the antibody forming tissues shortly after the time when endotoxin was injected. In subsequent experiments, when 10-μg amounts only of antigen were injected, endotoxin was given at the same time as the antigen.

THE EFFECT OF ENDOTOXIN WHEN INJECTED WITH H.HSA

(1) *On the weight of the draining lymph node*

Injection of endotoxin (40 μg) with H.HSA (10 μg) compared with injection of H.HSA alone caused an increase in the weight of the nodes (popliteal and aortic) which drained the injection site. The additional increase in the weight of the nodes was noticeable about 24 hours after injection of the antigen and was approximately constant at about 20 per cent during the following 2 days.

(2) *On the retention of the labelled H.HSA in the rat*

The amounts of radioactivity present in the footpads (site of injection), the popliteal and aortic lymph nodes and in the spleen, expressed as a percentage of the amount of radioactivity injected, are plotted in Fig. 2. Injection of endotoxin with labelled H.HSA

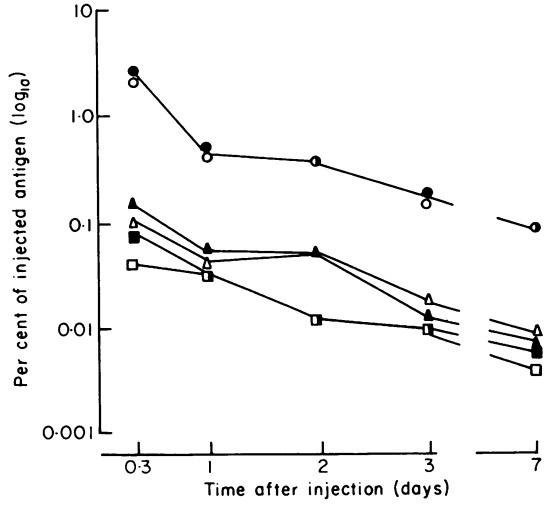

FIG. 2. The radioactivity (labelled H.HSA) present in rat tissues after the injection of labelled H.HSA (10 μg) with or without endotoxin (40 μg). Radioactivity present in hind footpads (○), popliteal and aortic lymph nodes (△) and spleen (□). The solid symbols indicate the values when endotoxin as well as H.HSA was injected.

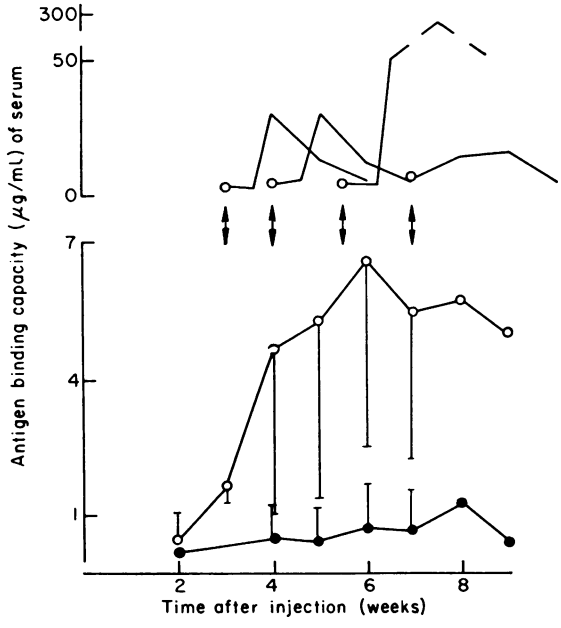

FIG. 3. The immune response in rats injected with H.HSA (10 μg) or a HSA–antibody complex and endotoxin (50 μg) and challenged at various times with HSA (1 mg). The curve (○) in the lower graph is the antibody response to the injection of H.HSA and endotoxin. The curves in the upper graph represent the antibody response when groups of four rats in the experiment were, at the time indicated by the double-headed arrows, injected with HSA. The second curve (●) in the lower graph is the antibody response to the injection of a precipitate of HSA and rat anti-HSA antibody (10 μg HSA) and endotoxin (50 μg). The vertical lines in the lower graph are the standard deviations of the means. They are arbitrarily drawn on one side only of the mean in order not to overload the graph with unnecessary lines.

did not affect appreciably the amount of labelled H.HSA present in these tissues at times up to 7 days after injection of H.HSA.

Similar experiments using unmodified HSA also failed to show any effect of endotoxin on its retention.

(3) *On the localization of labelled H.HSA in the popliteal and aortic lymph nodes*

Rats were injected with H.HSA (10 μg) with or without endotoxin (40 μg). Two rats from each group were killed and examined at 4, 8, 12, 18 and 48 hours after the injection, and sections of popliteal and aortic nodes were examined. No consistent differences were found in the localization patterns in nodes from the two groups. Each showed strong medullary localization of the antigen with very infrequent and, when present, very weak follicular localization.

(4) *On the production of antibody*

In one of three similar experiments H.HSA (10 μg) was injected into a group of twenty-four rats and a second group of twenty-four rats was injected with H.HSA (10 μg) and endotoxin (50 μg). All rats were bled weekly over a period of 9 weeks. At each of four intervals, four rats were removed from each group and injected into the hind footpads with 1 mg HSA in saline.

Rats injected with H.HSA alone did not produce antibody; rats injected with H.HSA and endotoxin did produce antibody. The mean serum antibody titres of this latter group are shown in Fig. 3, and indicate that a primary antibody response was obtained rising to a maximum level at 6 weeks after the injection of antigen. Three positive sera taken from rats 2 weeks after the initial injection of H.HSA and endotoxin were fractionated on Sephadex G-200. About 90 per cent of the antibody activity was recovered in the IgG region.

The response of rats in groups initially injected with H.HSA and endotoxin and subsequently with 1 mg HSA is also shown in Fig. 3. There was a wide variation in the behaviour of individual rats. In rats whose sera after the primary H.HSA injection had low antibody titres (<2·0 μg/ml), no secondary antibody response was obtained. Other rats produced high titres of antibody and this was most apparent in the group which was injected with HSA 6 weeks after the injection of H.HSA and endotoxin.

THE ANTIBODY RESPONSE OF RATS FOLLOWING THE INJECTION OF ANTIGEN–ANTIBODY MIXTURES AND ENDOTOXIN

Injection of HSA (10 μg) with varying amounts of specific antibody did not result in detectable antibody formation (Lang and Ada, 1967b). In the present experiments, endotoxin (50 μg/rat) was injected together with 10 μg HSA complexed with an estimated equivalent amount of antibody. Three experiments were carried out, In two, rat anti-HSA antiserum was mixed with either HSA or H.HSA and the mixture together with endotoxin injected immediately. In both cases, about half the rats formed small amounts of antibody (at 28 days, mean titres less than 2 μg/ml); slightly additional amounts only of antibody were formed when the positive rats were challenged with HSA (100 μg). In the third experiment, rats were injected with an HSA–anti-HSA precipitate washed free of soluble antibody. The resulting antibody response (Fig. 3) was only slightly greater. About half of the rats responded. When groups of these rats were challenged with 1 mg

HSA at times between 3–9 weeks after the primary injection, antibody titres in the serum decreased, and stayed low. In other experiments, it was shown that injection of endotoxin with the HSA–antibody complex affected neither the retention nor the pattern of localization of the labelled antigen.

INJECTION OF PRIMED RATS WITH HSA OR WITH AN HSA–ANTIBODY COMPLEX

A possible reason for the poor antibody response of rats injected with an antigen–antibody complex and endotoxin might have been an inhibitory effect by the added antibody (e.g. Uhr and Baumann, 1961). Since injection of antibody has been reported not to inhibit the secondary response, the efficiency of an HSA–antibody complex and of HSA were compared.

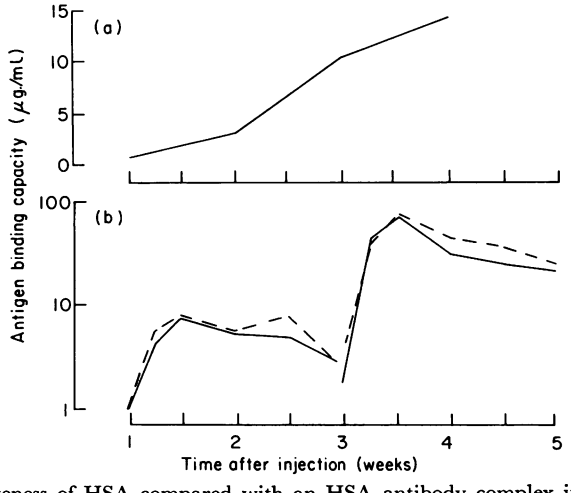

FIG. 4. The effectiveness of HSA compared with an HSA–antibody complex in the induction of a secondary antibody response in rats primed by a previous injection of H.HSA and endotoxin. (a) Primary response in rats injected with H.HSA (10 μg) and endotoxin (50 μg). (b) Responses due to challenge with 100 μg of HSA (———) or of HSA–antibody complex containing 100 μg HSA (– – – –).

Rats previously injected with H.HSA (20 μg) and endotoxin (50 μg) were challenged 1 and 3 weeks later with either HSA or a freshly prepared HSA–antibody complex. Each rat received in the second injection an estimated 100 μg of HSA. The results in Fig. 4 show that at both time points, the secondary response to an antigen–antibody complex or to free antigen were closely similar. In another experiment, groups of rats primed as above were challenged at 1 week with either HSA or an HSA–antibody mixture containing HSA labelled with ^{125}I. From each group, two rats were killed 24 hours later and one rat 4 days later. Other rats in each group were kept for antibody estimations. The popliteal and aortic lymph nodes were examined by radioautography. In those rats injected with the antigen–antibody complex, there was, as expected, strong medullary and follicular localization of the antigen at both time points. In rats injected with HSA alone, there was weak medullary and no detectable follicular localization of antigen at 24 hours but strong follicular localization at 4 days. Antibody titres in the two groups were equal at times up to 7 days after challenge. After this time, the antibody response of the rats challenged with HSA alone was slightly lower than in the other group. No antibody was detectable in sera taken at the time of challenge.

THE EFFECT OF ENDOTOXIN ON THE IMMUNE RESPONSE TO BACTERIAL FLAGELLIN

It was now of interest to see whether an increased response would be shown with a more potent immunogen than HSA. Rats (six per group) were injected at the same time either with endotoxin (50 μg) and 2 μg flagellin or with flagellin (2 μg) alone. The antibody titres produced when endotoxin was given were substantially higher and there was less variation between rats in a group than when flagellin alone was injected (Fig. 5).

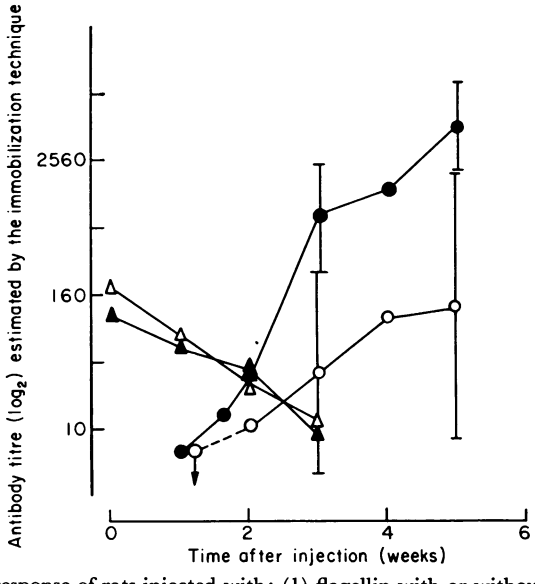

FIG. 5. The antibody response of rats injected with: (1) flagellin with or without endotoxin; and (2) a flagellin–antibody complex with or without endotoxin. Production of antibody after the injection of 2 μg flagellin alone (○) or with 50 μg endotoxin (●). Antibody present in the serum at different times after the injection of a flagellin (1 μg)—antibody complex alone (△) or with 50 μg endotoxin (▲). Vertical lines represent standard deviation of mean values.

It was previously shown that injection of rat anti-flagellin antibody with flagellin inhibited the primary response to this antigen (Lang and Ada, 1967a). This was repeated using a dose of antibody which was estimated from the immobilization titre just to react quantitatively with the antigen. Rats were injected either with the freshly prepared antigen–antibody mixture alone or with the freshly prepared antigen–antibody mixture and endotoxin. In both cases only the antibody injected with the antigen was detected in the serum, and this had a half-life in the blood of about 7 days (Fig. 5). Antibodies to flagellin prepared in rats precipitated very poorly when mixed with flagellin so an experiment to test the effect of endotoxin following an injection of a washed precipitated flagellin–antibody complex was not carried out.

DISCUSSION

The following observations were made when endotoxin (40–50 μg) was injected into the hind footpads of rats with antigen; either HSA, heat denatured HSA (H.HSA; 10–20 μg) or flagellin (1–2 μg).

(1) Antibody was formed in increased or detectable amounts in situations where it

was known that there was substantial capture of the antigen by the cells of the reticulo-endothelial system in lymphoid tissues; that is, when flagellin, heat denatured HSA (H.HSA) or an HSA–antibody complex was injected (see also Dresser, 1962; Bauer, Mathies and Stavitsky, 1963; Frei, Benacerraf and Thorbecke, 1965). Antibody was not formed in detectable amounts when HSA and endotoxin was injected into rats.

(2) As far as could be determined by radioactivity measurements and radioautography, the administration of the endotoxin did not influence the trapping, retention or localization of radio-isotope-labelled HSA after injection as HSA, H.HSA or HSA–antibody complex. This contrasts with the effect of complete Freund's adjuvant which much reduced the rate of drainage of labelled flagella from the injection site, and increased the retention and changed the localization pattern of this antigen in the draining lymph nodes (Lind, 1968), when compared with injections made in saline.

(3) As a stimulus to the production of antibody to H.HSA (10 μg), endotoxin had the greatest effect when injected at the same time as the antigen. In this particular circumstance then, it could be inferred that the induction process in antibody formation largely took place within a few hours after the injection of the H.HSA. As the retention of antigen in the popliteal and aortic lymph nodes and spleens of the rats was very poor, it seems likely that little, if any, of the antigen persisted in the lymphoid system beyond 3–4 days. Nevertheless the production of antibody was prolonged, maximum antibody titres being reached 6 weeks after injection of antigen. Two comments can be made: (a) The prolonged antibody response, if not due to persisting antigen, may have been due to an increase in long lived antibody-forming cells (Miller, 1964). (b) It could be suggested that iodide might not have been associated with an antigenic determinant of the HSA and was therefore not a valid marker; against this, radioactivity associated with specific antigen has been recovered from lymph nodes previously injected with HSA and anti-HSA antibody (Ada and Lang, 1966).

(4) The immune response induced by injection of endotoxin with H.HSA was demonstrated not only by the primary antibody response but also by the subsequent demonstration of a secondary antibody response; i.e. the priming of cells for a secondary antibody response occurred as well as a primary antibody response.

The fate of endotoxin within lymphoid tissues and the manner in which it causes enhanced antibody production to other antigens are not known in detail. It is known to react with macrophages and, as an antigen, it would have been taken up by the macrophages in the medulla of the lymph node. Endotoxin is known to induce a profound secondary nodular proliferation in the spleen (Ward, Johnson and Abell, 1959; Thorbecke, Asofsky, Hochwald and Siskind, 1962) so it is likely that the endotoxin was present also in the follicles of the lymph nodes, and possibly at the surface of the reticular cell. Though endotoxin may have stimulated phagocytosis, this was not reflected in an increased uptake of H.HSA in the node, although it is conceivable that such an increase may have been balanced by an increased rate of destruction. The increased weight of the nodes indicated activity of the endotoxin.

An aim of the investigation was to relate the localization pattern of an antigen in lymphoid tissues to a subsequent antibody response. An attempt to do this in terms of antigen localization in only some of the lymph nodes must be interpreted with caution. However, the nodes concerned (popliteal and aortic) retained more of the antigen than the spleen and probably more than other lymph nodes. Furthermore, the pattern of flagellar antigen localization in the spleen (Nossal, Austin, Pye and Abbot, 1966) is

basically similar to that in lymph nodes. With this reservation, the conclusions emerging from the study were:

(1) Follicular localization was not a requisite for either antibody production or the priming of cells for a subsequent secondary antibody response to occur.

(2) There was a correlation between the trapping of antigen in the lymph node medullary macrophages and subsequent antibody production (H.HSA and endotoxin versus HSA and endotoxin). Although trapping of antigen by medullary macrophages was not shown to be a necessary step, the finding was consistent with the work of others (quoted above) that interaction of antigen with macrophages was necessary for antibody production to occur.

(3) A secondary antibody response could be fired by antigen without its being extensively trapped in either the lymph node medullary macrophages or lymphoid follicles. Once specific antibody was present, however, strong follicular localization of the antigen occurred.

It is likely that the localization of antigen in the lymphoid follicles—a method of fixation of antigen by antibody to the reticular cell surface so that the availability of antigen in tissues for contact with appropriate cells is greatly prolonged—may be the body's most efficient mechanism for firing a secondary antibody response. White (1960) and Thorbecke *et al.* (1962) have suggested that the development of follicles in lymphoid tissue may be in preparation for the secondary antibody response. This would require that the lymphoid follicle was an area of traffic for memory (primed) cells. If this hypothesis is correct, the antibody response observed in cases where, after a single injection, antigen is found in both the medulla and follicles of lymphoid tissues may consist of a primary antibody response augmented by a secondary antibody response.

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