Immunology, 1970, **19**, 945.

The Morphology of Immune Reactions in Normal, Thymectomized and Reconstituted Mice

III. RESPONSE TO BACTERIAL ANTIGENS: SALMONELLAR FLAGELLAR ANTIGEN AND PNEUMOCOCCAL POLYSACCHARIDE

A. J. S. Davies, R. L. Carter, Elizabeth Leuchars, Valerie Wallis and F. M. Dietrich

Chester Beatty Research Institute, Institute of Cancer Research, Royal Cancer Hospital, Fulham Road, London S.W.3 and Biological Research Laboratories, Ciba Limited, Basel

(*Received* 23*rd March* 1970)

Summary. The response to two bacterial antigens has been studied in the lymph nodes draining their site of injection in normal mice, and in thymectomized, irradiated bone-marrow injected mice with and without a reconstituting thymus graft. A chromosome marker was used to differentiate between the response of cells derived from the bone-marrow and the thymus graft. Cells of thymic origin were stimulated to mitosis only to a slight extent by salmonellar flagella and not at all by pneumococcal polysaccharide. Histopathological changes in the regional lymph nodes were small compared with those previously studied (to sheep red blood cells and to oxazolone). Animals deficient in thymus cells were incapable of sustained antibody production after injection of salmonellar flagella but they were able to produce nearly normal amounts of circulating antibody against pneumococcal polysaccharide.

INTRODUCTION

Two previous papers have dealt with various aspects of the reactions in normal, thymectomized and reconstituted mice to immunization with sheep erythrocytes (Davies, Carter, Leuchars, Wallis, and Koller, 1969a) or sensitization with oxazolone (Davies, Carter, Leuchars and Wallis, 1969b). The present account is concerned with the responses evoked in similar groups of mice injected either with flagellar (H) antigen prepared from *S. typhi*, or with pneumococcal polysaccharide. These two antigens differ in many obvious respects (Wilson and Miles, 1964; Dubos and Hirsch, 1965). The first is a thermolabile antigen, probably composed of fibrous proteins of the myosin-keratin type. It appears to contribute nothing to the pathogenic effects of the intact microorganism. It is immunogenic but the antibodies which it evokes probably add little to the effective antibacterial immunity developed by the host. The capsular polysaccharide obtained from pneumococci contains no protein moiety and is the most important single factor in the pathogenic activity of the organism. Antibodies developed against pneumococcal polysaccharide generally provide effective antibacterial immunity though this substance is well known for the ease with which (under certain circumstances) it may induce tolerant rather than immune states (Felton, 1949; Chase, 1959).

Many aspects of the immune responses evoked by bacteria are still obscure but it is

becoming clear that cellular reactions of the delayed hypersensitivity type are often implicated in circumstances where humoral responses were hitherto thought to predominate (Mackaness, 1967). Reactions of this kind have been described in experimental infections with Salmonellae (Collins and Mackaness, 1968) and they may operate in pneumococcal infections as well (see Holborow and Loewi, 1967). The activities of thymus-derived and bone-marrow-derived lymphoid cells in immune responses to bacterial antigens have not been studied in detail and, in the present paper we have investigated these cells in CBA/H/CBA/H. *T6T6* radiation chimaeras.

MATERIALS AND METHODS

Mice

The experimental design was similar to that adopted in previous experiments in which the immune response to sheep erythrocytes and to oxazolone was analysed (Davies *et al.*, 1969a, b). CBA/H male mice were thymectomized at 8 weeks of age. 2 weeks later they received 850 rads total body irradiation from a 220 kV X-ray machine. Within 3 hours, an intravenous injection of 5×10^6 cells of syngeneic (CBA/H) bone marrow was given. Some of the mice were then grafted with a single lobe of a CBA/H. *T6T6* neonatal thymus under the capsule of the left kidney; approximately 180 animals were prepared in this way and they will be referred to in the text as reconstituted mice. A further 120 animals, which did not receive thymus grafts, will be referred to as deprived mice. A group of 120 normal males of the CBA/H strain was also studied. Full details of the preparation of CBA/H/CBA/H. *T6T6* chimaeras have been published elsewhere (see Davies, Leuchars, Wallis and Koller, 1966). In all instances a period of 50 days elapsed between the preparation of the chimaeras and their subsequent immunization; the group of normal mice was also left for 50 days so that all the animals were of comparable chronological age when they were injected with antigen.

Salmonellar flagellar antigen

A preparation of purified *S. typhi* H (i) antigen was diluted in sterile distilled water to a concentration of 230 μg/ml and stored at $-40°$ in sealed glass tubes until required. It was then diluted ten-fold with sterile saline and injected into the forepaws and subcutaneously into two sites high on the back of each mouse—an anatomical distribution of antigen which is known to stimulate both internal and external axillary lymph nodes. The injection volume (0·1 ml) was divided equally between the four points of injection and the total amount of antigen injected into each mouse was 2·3 μg. This dose of antigen had previously been shown to be the minimum required to establish a vigorous and reproducible immune response as measured by antibody production. Five mice were bled in each group on each of the 10 days following immunization. Individual sera were diluted in physiological saline in Takatsy microtitrator tiles (Shandon Scientific Co.). An agglutinable suspension of the appropriate Salmonella organisms ($1\cdot25\times10^9$ organisms/ml) was added to the diluted antisera and the mixture was left at room temperature for 2–3 hours. The end-points were then determined by examining the agglutinates under a dissecting microscope.

Pneumococcal polysaccharide

Highly polymerized SIII pneumococcal polysaccharide was suspended in saline; 1 μg in a total volume of 0·1 ml was distributed equally among four injection sites in each animal

in the same manner and in the same number of mice as the salmonellar flagellar antigen.

Blood was removed from individual mice on each of the ten days after immunization. The sera were titrated in micro titrator tiles. Glutaraldehyde-treated mouse erythrocytes (Bing, Weyand and Stavitsky, 1967), sensitized with pneumococcal polysaccharide, were used as marker cells in a passive agglutination technique.

Cytology

On each of the 10 days following introduction of antigen into reconstituted mice, three animals were injected with colcemid 1½ hours before they were killed by cervical dislocation. The axillary lymph nodes were removed, and their constituent cells prepared for cytological analysis by a technique basically the same as that described by Ford (1966).

Histopathology

On each of the 10 days following introduction of antigen into normal, deprived and reconstituted mice, five animals were weighed and killed. Their axillary lymph nodes were weighed and fixed in Bouin's solution. Paraffin sections were prepared at 5 μ and stained with haematoxylin and eosin. Ten uninjected mice were examined in each of the three experimental groups. Half of them were killed at the time the other mice were immunized. The other half were killed 10 days later at the end of the experiment. The slides were coded and examined independently, as were the chromosome preparations.

RESULTS

I. RESPONSE TO SALMONELLAR FLAGELLAR ANTIGEN

Serology. Titres of agglutinins formed in normal, deprived and reconstituted mice against salmonellar flagellar antigen are shown in Fig. 1. The early stages of the serological response were similar in all three groups, rising slowly on days 1 and 2, and then increasing steeply to reach a peak on days 4 and 6. Obvious differences in response emerged subsequently. Antibody titres in normal and reconstituted mice remained elevated but levels in deprived mice rapidly declined.

In previous investigations of the immune reaction evoked by sheep erythrocytes and oxazolone (see introduction), a period of 10 days was found to be sufficient to cover most aspects of the primary response. A longer period of time seemed to be indicated for studying the reaction of salmonellar flagellar antigen, and further observations were accordingly made. In a separate experiment, 2·3 μg salmonellar flagellar antigen was injected intraperitoneally into forty-five mice (fifteen normal, fifteen deprived and fifteen reconstituted). The antibody response was followed at intervals for more than 5 weeks and a second intraperitoneal injection of antigen was given on day 38 in order to study the course of the secondary response. Antibody titres found in the three groups are recorded in Fig. 2. The primary response during the first 10 days was smaller than that provoked by the same dose of salmonellar flagellar antigen injected subcutaneously, though the overall pattern of the three curves was similar. The subsequent decline in antibody levels in deprived mice, observed in the earlier experiment, could be followed over a longer period and it was clear that there was a profound difference in response between deprived animals on the one hand, and normal or reconstituted mice on the other. Rechallenge at day 38 provoked an abrupt increase in antibody levels in all three groups, but the response in deprived

animals was weaker and less well sustained than that seen in normal and reconstituted mice. Some of the sera were analysed by differential ultracentrifugation on sucrose gradients to determine the proportion of 19S (IgM) and 7S (IgG) antibody to salmonellar flagellar antigen. The results were not wholly satisfactory as separation into two classes was not complete on all the gradients. It was, however, clear that in deprived mice there was a quantitative deficiency of 7S antibody. This matter is the subject of further investigation.

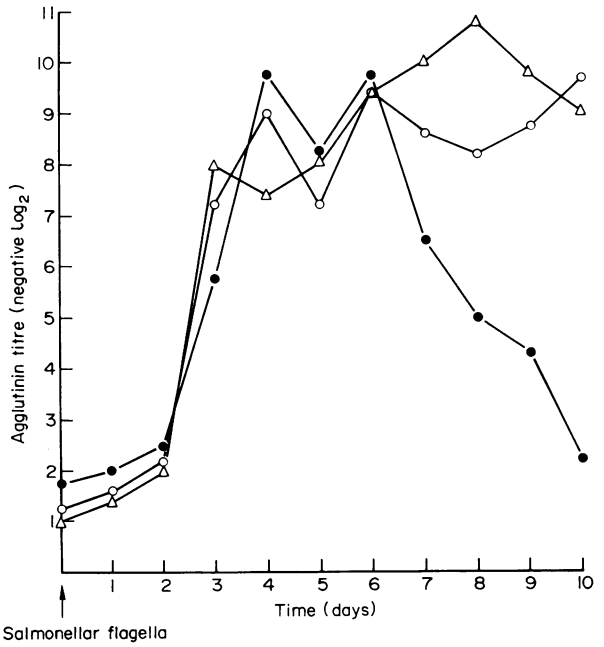

FIG. 1. The agglutinin titres of normal (△), deprived (●) and reconstituted (○) mice (see text) in relation to time after subcutaneous injection of 2·3 μg of salmonellar flagellar antigen.

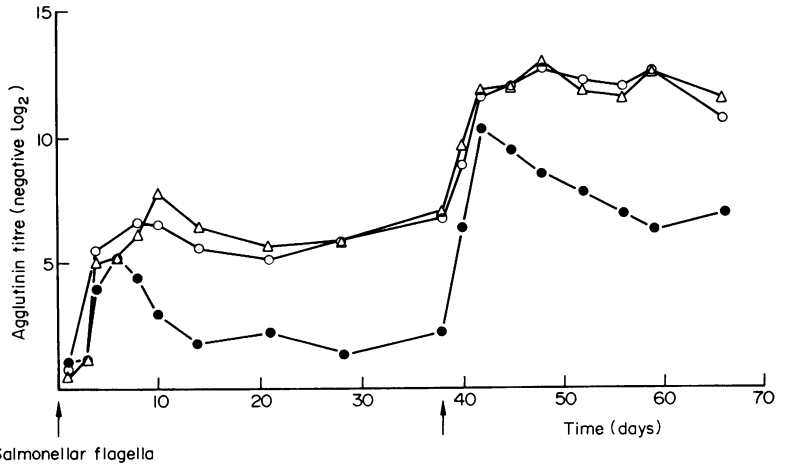

FIG. 2. The agglutinin titres of normal (△), deprived (●) and reconstituted (○) mice (see text) in relation to time after one and two intraperitoneal injections of 2·3 μg of salmonellar flagellar antigen.

TABLE 1

CYTOLOGIC ANALYSIS OF THE MITOTIC RESPONSES IN THE DRAINING LYMPH NODES OF RECONSTITUTED MICE TO AN INJECTION OF SALMONELLAR FLAGELLA; FOR COMPARISON THE RESPONSES TO SHEEP RED BLOOD CELLS (5×10^8) CELLS AND OXAZOLONE (3 PER CENT SOLUTION IN ABSOLUTE ALCOHOL) ARE GIVEN

Day after injection	Total No. of cells scored	Percentage known thymus-derived cells	No. of lymph nodes examined
1	0	0	6
2	33	57·6	6
3	79	7·6	8
4	57	24·6	6
5	1	0	4
6	0	0	6
7	41	12·2	6
8	0	0	6
9	0	0	6
10	0	0	6
Control uninjected	49	32·7	28
SRBC (day 3)	664	59·6	12
Oxazolone (day 3)	579	77·7	12

Cytology. Results relating to the proliferation of thymus-derived cells (carrying the *T6T6* marker) in reconstituted animals are shown in Table 1. Few mitoses were seen in axillary lymph nodes from untreated control mice and it is reasonable to assume that mitosing cells in nodes from test mice had been brought into division as a result of immunization. The percentage of dividing cells arising from the marked thymus graft is plotted separately in Fig. 3. It is clear from Table 1 and Fig. 3 that there was a short burst of mitotic activity among thymus-derived cells during the early stages of the immune response, but it was small compared to the responses evoked by sheep erythrocytes or oxazolone. This is emphasized more strongly if the thymus-derived cells are recorded as absolute numbers of cells per lymph node rather than as percentages (cf. Carter, Davies, Leuchars, Wallis and Gershon, 1969): considered in this fashion, the response of thymus-derived cells to

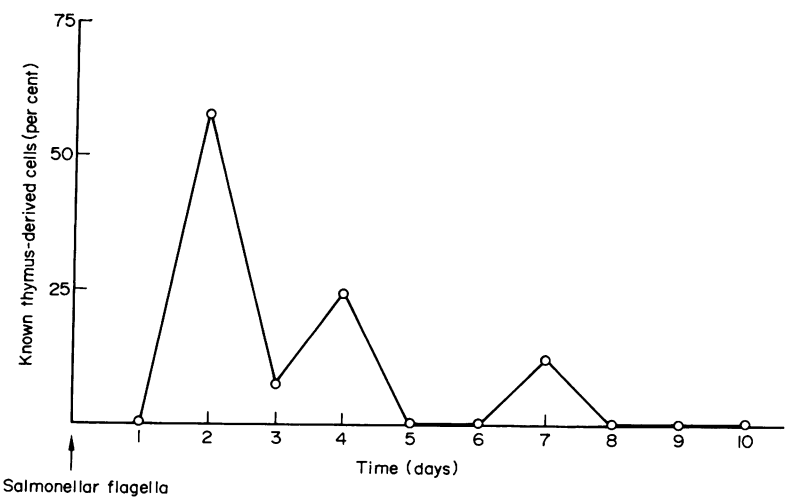

FIG. 3. The percentage of the dividing cell population known to derive from the thymus graft in the draining lymph nodes of reconstituted mice (see text) during a 10-day period after injection of salmonellar flagella.

salmonellar flagellar antigen was less than 10 per cent of that previously observed in relation to sheep cells or oxazolone.

Proliferation of thymus-derived cells in reconstituted mice was followed by a short period of mitotic activity among bone-marrow-derived cells. This, too, was small and short-lived.

Pathology. Lymph node and body weights recorded in all experimental animals were unremarkable. As in previous investigations, normal and reconstituted mice weighed more than deprived mice but there was no consistent change in body weight after immunization with salmonellar flagella in any of the groups. Changes in lymph node weight were similarly inconstant and nothing could be deduced from lymph node/body weight ratios.

Histological changes were examined in 482 axillary lymph nodes from the three test groups—192 from fifty normal mice, 131 from thirty-nine deprived mice and 159 from forty-four reconstituted mice. A further ninety-eight lymph nodes were examined from twenty-six uninjected mice. A definite morphological response developed in the three test groups though, in most instances, the reactions were moderate in extent and were not found in all the axillary nodes removed from any one animal at a given time.

Uninjected mice. Lymph nodes from uninjected normal or reconstituted mice were of normal cellularity. The primary follicles were small, germinal centres if present were poorly developed, the paracortical region contained mainly small lymphocytes, and the medullary cords were inconspicuous. Nodes from uninjected deprived mice were small, mainly as a result of paracortical hypoplasia; the absence of the usual population of lymphocytes in

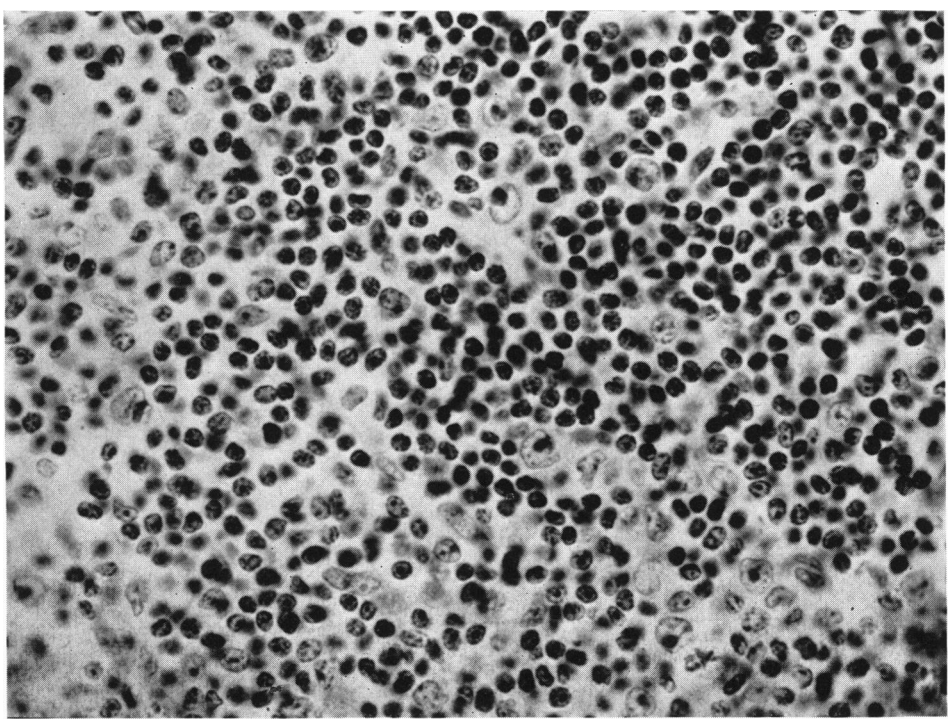

FIG. 4. Axillary lymph node; normal mouse; 3 days after injection of salmonellar flagella. View of paracortical region, showing moderate numbers of blast cells. Haematoxylin and eosin, × 675.

this zone revealed and possibly promoted large cells resembling histiocytes. Inactive follicles were occasionally seen and small medullary cords could be made out in a few nodes.

Normal mice. Changes in the lymph nodes were first seen on day 2 when paracortical blast cells were slightly increased in number (Fig. 4); other structures in the lymph nodes were normal at this time. Signs of proliferative activity in the paracortex were slight and, occasionally, confined to only one of the four axillary nodes removed from each mouse. By four days, the paracortical zones were again normal but some changes were becoming apparent in other regions of the nodes. The follicles were moderately increased in number and size, with large, active-looking germinal centres (Fig. 5). The medullary cords in some nodes were slightly enlarged and contained an increased proportion of plasma cells. The changes in the follicles and medullary cords persisted and were still apparent at 10 days but they were never more than moderately intense and, as with the earlier

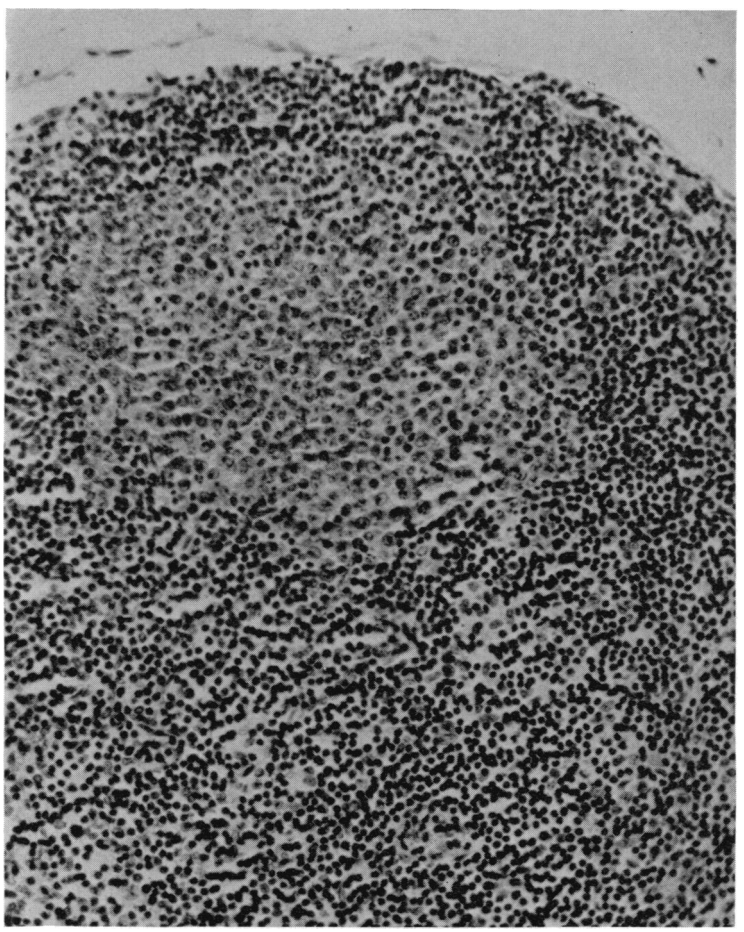

FIG. 5. Axillary lymph node; normal mouse; 5 days after injection of salmonellar flagella. Enlarged germinal centre in a superficially-placed follicle, which is poorly demarcated from the adjacent cortex. Haematoxylin and eosin, ×270.

paracortical reaction, were usually confined to (or most marked in) one of the group of four axillary nodes removed from each animal.

Deprived mice. Nodes from injected deprived mice showed paracortical hypoplasia comparable to that seen in uninjected deprived animals; this hypoplasia persisted unchanged throughout the experiment. The follicles increased in number and size on day 4 and some of them developed small active-looking germinal centres (Fig. 6). Moderate plasmacytosis in the medullary cords was seen in a few mice on and after day 5. These various changes were short-lived, declining rapidly after day 7, and tended to involve only one or two of the group of draining nodes which was examined.

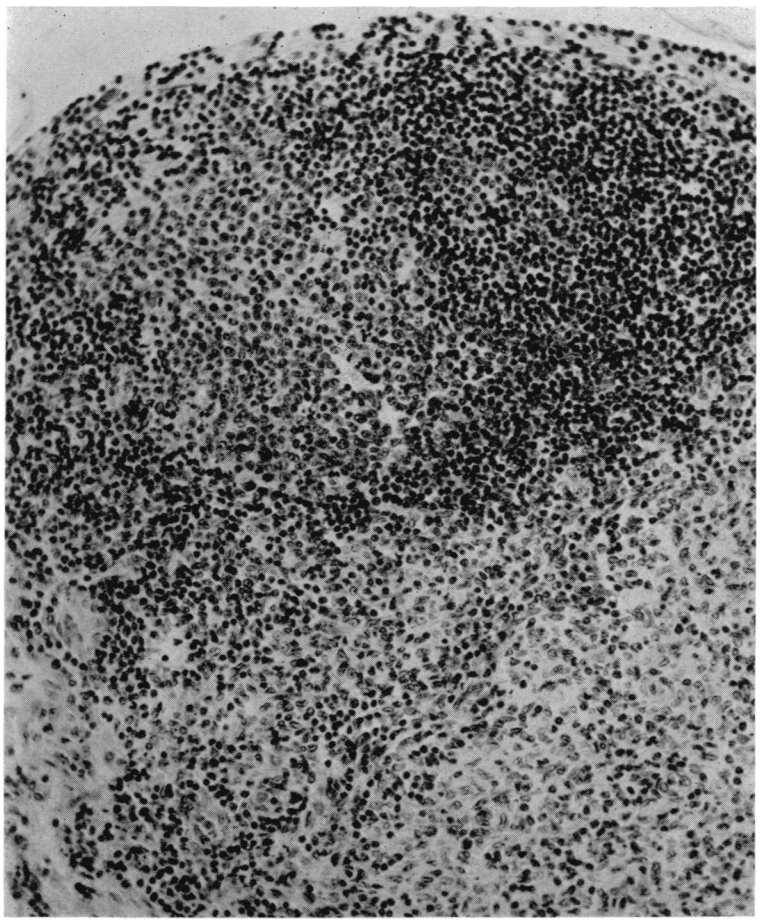

FIG. 6. Axillary lymph node; deprived mouse; 5 days after injection of salmonellar flagella. Enlarged germinal centre. Note hypoplasia of adjacent paracortex. Haematoxylin and eosin, ×270.

Reconstituted mice. The morphological response of nodes from reconstituted injected animals was similar to that previously described in normal injected mice. A few cells in mitosis were seen in the paracortex on day 2 but the increase in the number of paracortical blast cells was very slight. Enhanced follicular activity was apparent on day 3 and, accompanied by mild plasmacytosis in the medullary cords, persisted until day 7. Activity then declined (cf. nodes from normal mice) and normal appearances were restored by day 10.

II. RESPONSE TO PNEUMOCOCCAL POLYSACCHARIDE

Serology. Titres of antibodies found against pneumococcal polysaccharide are shown in Fig. 7. The titres obtained were low and rose late compared with the values observed by Howard, Elson, Christie and Kinsky (1969), using the same immunizing antigen but a different route of introduction and a somewhat different titration system. From the present experiments the most useful finding was that all groups of mice produced approximately the same response. There was thus no evidence of thymus-dependency in the immune response to pneumococcal polysaccharide.

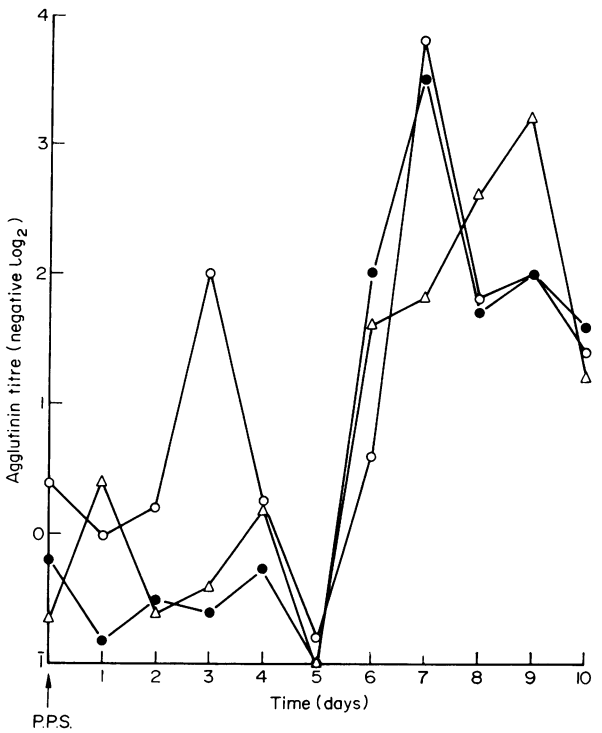

FIG. 7. The titres of anti-pneumococcal polysaccharide antibodies in normal (△), deprived (●) and reconstituted mice (○) (see text) at various times after subcutaneous injection of 1 μg of pneumococcal polysaccharide (P.P.S.).

On two counts, however, we did not think these results satisfactory: firstly, there was a tendency for unsensitized glutaraldehyde treated cells to agglutinate in the presence of antipneumoccoccal polysaccharide antibody; secondly, the titres obtained were low. Accordingly fresh groups of normal, deprived, and reconstituted mice were prepared. They were injected with 0·1 μg of SIII polysaccharide intravenously and the serum antibodies titrated 20 days later. The mean titres for the three groups at this time were, respectively, (neg. $\log_2$) 4·3, 3·0 and 5·0. None of these values was deemed different from any of the others. The titrations were very kindly carried out by Dr J. G. Howard of the Wellcome Research Laboratories, Beckenham, using a passive agglutination technique (Howard *et al.*, 1969). This experiment substantiates our earlier findings. It carries with it, however,

the hint that deprived mice may have a slightly subnormal response to injection of pneumococcal polysaccharide.

Cytology and pathology. The subsequent cytological and morphological findings were virtually negative. The cytological analysis was carried out in 138 nodes, two slides being prepared from each node. The results, shown in Table 2, indicate that there was neither suppression nor enhancement of mitotic activity after injection of pneumococcal polysaccharide. No consistent changes in body weight, lymph node weight, or lymph node/body weight ratio were seen, and no histological changes suggesting an immunological response developed in axillary lymph nodes from test animals 1–10 days after immunization.

TABLE 2

CYTOLOGIC ANALYSIS OF MITOTIC RESPONSES IN THE DRAINING LYMPH NODES OF RECONSTITUTED MICE TO AN INJECTION OF PNEUMOCOCCAL POLYSACCHARIDE

Day after injection	Injected*		Uninjected*	
	Geometric mean of No. of cells scored per lymph node	Percentage known thymus-derived cells	Geometric mean of number of cells scored per lymph node	Percentage known thymus-derived cells
1	4	15·3	3	6·7
2	2	36·8	2	26·3
3	3	15·8		
4	9	21·1	3	40·0
5	2	26·1	4	33·3
6	3	32·6	4	14·3
7	5	16·9	9	13·1
8	2	38·1	4	20·8
9	2	25·0	2	10·8
10	1	22·2		

* Ninety lymph nodes were examined from injected mice and forty-eight from uninjected mice.

DISCUSSION

In a detailed study of antibody production in young hybrid C3H/C57Bl mice after thymectomy, Humphrey, Parrott and East (1964) examined the response to five antigens: sheep erythrocytes, salmonellar H and O antigens, type 3 pneumococcal polysaccharide and haemocyanin. Of the five antibody responses tested, only one (that evoked by sheep erythrocytes) was consistently and markedly impaired in thymectomized animals. The serological reactions to the other antigens in thymectomized mice were strikingly variable with titres in several animals equal to or even exceeding those found in intact controls. The current series of experiments with CBA/H—CBA/H. *T*6*T*6 radiation chimaeras provides some additional information.

(a) *Response to salmonellar flagellar antigen.*

The antibody response to salmonellar antigen was not as variable as that observed in much younger mice by Humphrey and his colleagues. In our mice one injection of salmonellar flagellar antigen evokes an immune response which is only partially dependent upon the presence of a functioning thymus. For the first 6 days after immunization, the emerging antibody pattern is virtually indistinguishable in normal, reconstituted or thymectomized mice; this pattern is not altered qualitatively if the antigen is administered

intraperitoneally instead of subcutaneously. Evidence of thymus dependency becomes apparent only after peak antibody titres are reached on day 6, when it declares itself in two ways. (i) The primary antibody response in deprived animals is not sustained, and titres quickly fall back to low levels. (ii) The response to subsequent re-immunization is impaired, so that the secondary increase in antibody titre is smaller and more transient than that seen in normal or reconstituted mice which have been rechallenged. It seems that thymectomized mice can initiate an immune response to salmonellar flagellar antigen but cannot sustain it. This pattern of thymus-dependency has not been noted previously and the underlying mechanism is still obscure.

The cytological findings in reconstituted animals indicate that salmonellar flagellar antigen does evoke some proliferation among thymus-derived cells but the increase in mitotic activity is slight and short-lived. There are a few proliferating blast cells in the paracortex in the draining lymph nodes of normal and reconstituted mice; follicular hyperplasia and medullary plasmacytosis subsequently develop in all groups.

(*b*) *Response to pneumococcal polysaccharide.*

The antibody response studied here appears to be independent of normal thymic function. No increase in mitotic activity was demonstrated among thymus-derived cells in reconstituted mice, and antibody titres in deprived animals were similar to those found in normal or reconstituted mice. The regional lymph nodes showed no reactive changes at any time in any of the experimental groups. Previous investigators (Parrott and de Sousa, 1966) found that pneumococcal polysaccharide induced definite activation of follicles and medullary cords in draining lymph nodes from normal and thymectomized C3H/Bi mice although there was no blast cell reaction in the paracortex; the reasons for the present discrepancy are not clear. The anatomical localization of the immune response in our mice remains obscure; the most likely site is the spleen and further investigations will be made to support this suggestion.

Our current series of investigations into the morphology of the responses to sheep red blood cells, oxazolone and to bacterial antigens indicate that the mitotic response among thymus-derived cells varies considerably with the different antigens tested.

The present findings show that salmonellar flagellar antigen evokes little mitotic activity and that pneumococcal polysaccharide evokes none at all. There are a number of possible explanations for this lack of response. First, the healthy animal may carry several strains of pneumococci and salmonellae as part of its normal microflora. There is little information on commensal micro-organisms in the mouse and it is not clear to what extent such organisms induce immunity in their host. But it is possible that the poor response to salmonellar and pneumococcal antigens is due to some measure of pre-existing immunity to the same or related cross-reacting antigens. That this might impinge on mitotic activity in thymus-derived cells is suggested by experiments (Gershon, Wallis, Davies and Leuchars, 1968) which showed that thymus-derived cells do not respond by mitosis after repeated stimulation with the same antigen. Alternatively, antibacterial immunity may involve little or no contribution from thymus-derived cells. Baker and Landy (1966) have found that antibody production can be detected within 4–8 hours after mice were injected with pneumococcal polysaccharide. With such a brief inductive phase, there would hardly be sufficient time for thymus-derived cells to divide and participate in any immune response. It is known (Doenhoff, Davies, Leuchars and Wallis, 1970) that deprived mice have a small residuum of cells from the thymus: part of it is provided by thymus-derived cells which

'contaminate' the bone marrow inoculum used in the partial repair of the immune paresis induced by thymectomy and X-irradiation, and part represents a residual host population. It is possible that the remnant of cells, though shown to be inadequate to react effectively to sheep erythrocytes or to oxazolone (Davies *et al.*, 1969a, b), is nevertheless sufficient to potentiate the immune responses evoked by the two bacterial antigens. But this point cannot be resolved until 'super-deprived' mice are available which lack even this small residue of thymus-derived cells.

The obvious thymus-dependency of the *late* humoral antibody response to salmonellar flagellar antigen is not, however, clarified by these arguments. No further conclusions can usefully be drawn until definitive experiments have been made to determine whether co-operation between bone marrow and thymus cells does indeed occur, and whether (if it does) such co-operation is similar to that recorded in the development of humoral immunity to sheep erythrocytes.

ACKNOWLEDGMENTS

This investigation was supported by grants to the Chester Beatty Research Institute (Institute of Cancer Research; Royal Cancer Hospital) from the Medical Research Council, and the British Empire Cancer Campaign for Research.

The authors are grateful to a number of friends and colleagues for their help: to Professor B. A. D. Stocker, then of the Lister Institute of Preventive Medicine, for a gift of salmonellar flagellar antigen, Dr J. H. Humphrey of the National Institute for Medical Research for a gift of pneumococcal polysaccharide, Mrs I. Batty of the Wellcome Research Laboratories for the gift of a suspension of Salmonellae, Dr J. G. Howard of the Wellcome Research Laboratories for titration of anti-pneumococcal polysaccharide antibodies. We are also grateful to Professor P. C. Koller for his friendly advice and encouragement throughout the work. This and the previous two papers in the series were initiated after discussion with Dr D. M. V. Parrott of the University of Glasgow. We are most grateful to her for the suggestion and continued good advice.

REFERENCES

BAKER, P. and LANDY, M. (1966). 'Absence of a significant inductive phase in the immune response of the mouse to capsular polysaccharide antigens.' *J. Retic. Soc.*, **3**, 361.

BING, D. H., WEYAND, J. G. M. and STAVITSKY, A. B. (1967). 'Hemagglutination with aldehyde-fixed erythrocytes for assay of antigens and antibodies.' *Proc. Soc. exp. Biol. (N.Y.)*, **124**, 1166.

CARTER, R. L., DAVIES, A. J. S., LEUCHARS, E., WALLIS, V. J. and GERSHON, R. K. (1969). 'The mitotic response of thymus-derived cells to a variety of different antigens.' *Lymphatic Tissues and Germinal Centres in Immune Response* (Ed. by L. Fiore-Donati and M. G. Hanna, Jr), p. 143. Plenum Press, New York.

CHASE, M. W. (1959). 'Immunologic tolerance.' *Ann. Rev. Microbiol.*, **13**, 349.

COLLINS, F. M. and MACKANESS, G. B. (1968). 'Delayed hypersensitivity and Arthus reactivity in relation to host resistance in Salmonella-infected mice.' *J. Immunol.*, **101**, 830.

DAVIES, A. J. S., CARTER, R. L., LEUCHARS, E., WALLIS, V. J. and KOLLER, P. C. (1969a). 'The morphology of immune reactions in normal, thymectomized and reconstituted mice. I. The response to sheep erythrocytes.' *Immunology*, **16**, 57.

DAVIES, A. J. S., CARTER, R. L., LEUCHARS, E. and WALLIS, V. J. (1969b). 'The morphology of immune reactions in normal, thymectomized and reconstituted mice. II. The response to oxazolone.' *Immunology*, **17**, 111.

DAVIES, A. J. S., LEUCHARS, E., WALLIS, V. and KOLLER, P. C. (1966). 'The mitotic response of thymus-derived cells to antigenic stimulus.' *Transplantation*, **4**, 438.

DOENHOFF, M. J., DAVIES, A. J. S., LEUCHARS, E. and WALLIS, V. (1970). 'The thymus and circulating lymphocytes of mice.' *Proc. roy. Soc. B.*, (in press).

DUBOS, R. J. and HIRSCH, J. G. (1965). *Bacterial and Mycotic Infections of Man*, 4th edn. Lippincott, Philadelphia.

FELTON, L. D. (1949). 'The significance of antigen in animal tissues.' *J. Immunol.*, **61**, 107.

FORD, C. E. (1966). 'The use of chromosome markers.' *Tissue Grafting and Radiation* (Ed. by H. S. Micklem and J. F. Loutit), Appendix I, p. 197. Academic Press, New York.

GERSHON, R. K., WALLIS, V., DAVIES, A. J. S. and LEUCHARS, E. (1968). 'Inactivation of thymus cells after multiple injections of antigen.' *Nature* (*Lond.*), **218**, 380.

HOLBOROW, E. J. and LOEWI, G. (1967). 'Delayed hypersensitivity and polysaccharide-containing antigens.' *Brit. med. Bull.*, **23**, 72.

HOWARD, J. G., ELSON, J., CHRISTIE, G. H. and KINSKY, R. G. (1969). 'Studies on immunological paralysis. II. The detection and significance of antibody-forming cells in the spleen during immunological paralysis with type III pneumococcal polysaccharide.' *Clin. exp. Immunol.*, **4**, 41.

HUMPHREY, J. H., PARROTT, D. M. V. and EAST, J. (1964). 'Studies on globulin and antibody production in mice thymectomized at birth.' *Immunology*, **7**, 419.

MACKANESS, G. B. (1967). 'The relationship of delayed hypersensitivity to acquired cellular resistance.' *Brit. med. Bull.*, **23**, 52.

PARROTT, D. M. V. and DE SOUSA, M. A. B. (1966). 'Changes in thymus-dependent areas of lymph nodes after immunological stimulation.' *Nature* (*Lond.*), **212**, 1316.

WILSON, G. S. and MILES, A. A. (1964). *Topley and Wilson's Principles of Bacteriology and Immunity*. Arnold, London.