Immunoglobulins:

Cell Bound Receptors and Humoral Antibodies

Volume 26

Organized by:

R. E. BALLIEUX

M. GRUBER

H. G. SEYEN

FEBPBY

FEDERATION OF EUROPEAN BIOCHEMICAL SOCIETIES
EIGHTH MEETING, AMSTERDAM, 1972

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R. E. BALLIEUX, *Utrecht*
M. GRUBER, *Groningen*
H. G. SEIJEN, *Groningen*

1972

NORTH-HOLLAND / AMERICAN ELSEVIER

ISBN North Holland: 0 7204 4126 9

Publishers:

North-Holland Publishing Company - Amsterdam - London

Sole distributors for the USA and Canada:

American Elsevier Publishing Company, Inc.
52 Vanderbilt Avenue
New York, N.Y. 10017

ERRATUM

Volume 26

The ISBN number has been changed into:

ISBN North-Holland Series: 07204 4300 8
Volume: 07204 4326 1

Printed in The Netherlands

INTRODUCTION

The progress in understanding the biology and chemistry of the immune response in recent years is reflected in a large number of specialized meetings. We felt that a symposion forming part of a general biochemical meeting should have a general character. We, therefore, invited lecturers, who together cover a broad range. At the same time, we considered it useful to bring together lecturers on the cellular processes as well as on the structural aspects of immunoglobulins and antigens.

Rapid publication was considered to be of primary importance and precluded editing. As a result some duplication and heterogeneity in presentation has to be accepted.

October 1972

The Organizers.

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DYNAMICS OF IMMUNOGLOBULIN FORMING CELLS AND THEIR PRECURSORS

F.J. KEUNING
Department of Histology, R.U. Groningen
The Netherlands

PERIPHERAL LYMPHOID TISSUE

The immunologically responsive tissue is the *peripheral* lymphoid tissue as found in lymph nodes, spleen, tonsils, Peyer's patches and appendix. These peripheral lymphoid organs have markedly corresponding patterns of basic structures (follicles etc.) and they are subject to an equally corresponding lymphoid cell traffic. This traffic consists of lymphoid cells continuously being delivered to these organs by the blood stream and continuously being carried off, mostly via the lymph stream, to be returned to the blood circulation.

Such differences in anatomical structure as do exist between these organs mainly reflect the differences in the mode of entry of antigens: the afferent lymph stream in lymph nodes, the blood in the spleen and the mucosal epithelium in the tonsils, Peyer's patches and appendix.

Consequently the peripheral lymphoid organs can be considered the meeting point between immunologically competent lymphoid cells and antigens entering the organism via various routes.

T- AND B-CELLS

Among the lymphoid cells populating these peripheral lymphoid organs two main classes, T- and B-cells, can be distinguished according to their *central* lymphoid tissue of origin.

T-cells are small lymphocytes of thymus origin. In the thymus they arise by active multiplication from bone marrow derived stem cells. T-cells are long-lived, recirculating lymphocytes; their repeated exchange between blood and peripheral lymphoid organs results in a continuous redistribution over these organs (Gowans, 1959). In the lymphoid organs the T-cells populate clearly defined so-called "thymus-dependent areas". Histologically these T-cell regions in peripheral lymphoid organs (except the spleen) are characterized by the presence of epitheloid venules, the walls of which always contain numerous lymphocytes leaving the blood stream. T-cells represent the immunocompetent elements for all cellular types of immune response, and their descendants.

B-cells, the second category of lymphoid cells are derived from the bone marrow and via the blood stream directly fed into peripheral lymphoid organs. They have been shown to represent the precursors of antibody forming cells (Miller and Mitchell, 1969). The natural history of the B-cell population in peripheral lymphoid tissue, however, is more complicated and much less precisely known. B-lymphocytes and their descendants also populate well defined regions in all peripheral lymphoid organs viz. the lymphoid follicles with the adjoining so-called marginal zones.

The delivery by the blood of T- and B-lymphocytes to thymus dependent areas and follicles respectively is easily seen, microscopically, 24 hrs. after *local* irradiation of a lymph node, the spleen or the appendix, in which the radiation has destroyed all preexistent lymphoid cells (Bos 1967).

LYMPH NODE FUNCTION

The above mentioned aspects of the functional anatomy of peripheral lymphoid tissue are shown in figure 1 for the lymph node. In any lymph node antigenic stimulation is achieved by introduction of antigen into connective tissue regions draining upon that node. The antigen is carried along with the lymph which enters the lymph node by the afferent lymph vessels and spreads in the subcapsular sinus surrounding the whole cortex of the lymph node. From here the lymph passes through the lymphoid tissue, first of the outer cortex (B-cell region), then through the paracortical areas (T-cell region) and subsequently into the medulla from where it is carried off towards the venous blood stream by the efferent lymph vessel.

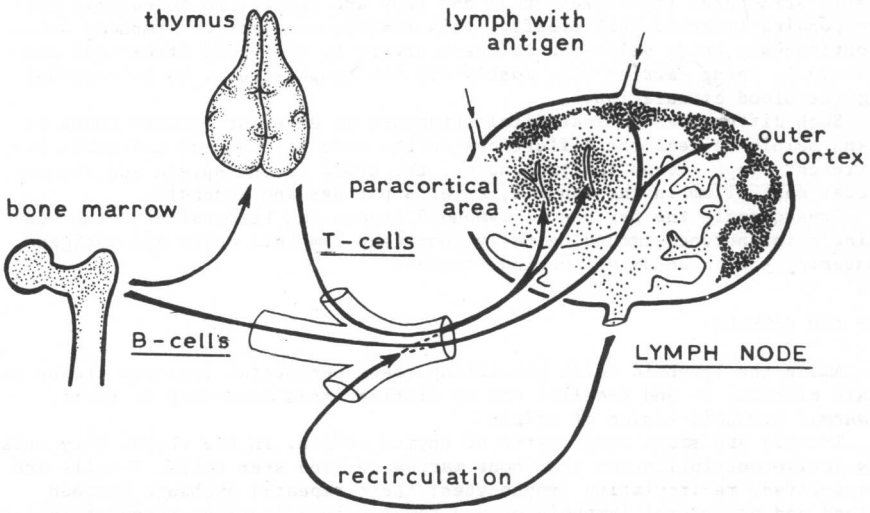

Fig. 1. Cell traffic in lymph node: delivery of bone marrow- and thymus-derived lymphocytes to outer cortex and paracortical area respectively, and recirculation of lymphocytes. Antigen brought in with lymph.

Antigen was shown to be retained in the lymph node in three different localizations (Nossal et al., 1968). The majority is phagocytized and metabolized by macrophages, especially those of the sinuses; it is not yet clear whether this antigen still plays a role in the immune response under normal conditions. Another, small, part of the antigen is trapped, apparently without being phagocytized, in the outer cortical regions most probably along the cell membranes of dendritic reticular cells present between the lymphoid cells. Trapped antigen is found in this location for only a few hours after antigen administration. Parallel with the disappearance from here, antigen was found to localize, equally without being phagocytized, in the centres of the follicles where it may be retained for weeks.

IMMUNE RESPONSE

The immune response elicited by the antigen essentially comprises three distinct processes: a lymphoblastic response of T-lymphocytes in the paracortical areas (i), a plasmacell response of B-cells present in the outer cortex (ii), and a blast cell response, equally of B-cells, in the follicular centres representing the well known germinal centre activity (iii).

(i) Histologically the first T-cell derived blast type cells appear in the paracortical areas 24-36 hrs. after antigen administration. These cells thereupon start considerable mitotic activity; the end-cells of the process are small lymphocytes which are added to the recirculating T-cell population. These cells are responsible for the specificity of cell-mediated immunological reactions like allograft rejection, contact sensitivity etc. In addition the blastcell response of T-cells plays an essential role -called "helper function"- in starting certain plasmacell responses which consequently have been designated as T-dependent (see below).

(ii) The plasmacell response represents antibody formation. Upon appropriate antigenic contact, for which the assistance of a T-cell response may or may not be needed, precursor cells of the B-lineage, present in the interfollicular (marginal zone) of the outer cortex and the lymphocyte mantles of the follicles, start transforming into large basophilic plasmablasts (Veldman, 1970). With thymus-*independent* antibody formation the first plasmablasts are seen in the outer cortex of the lymph node some 18-24 hrs. after the antigen.

Each of these newly arisen plasmablasts, by multiplication and differentiation (immature plasma cells), gives rise to a clone of mature plasma cells (see fig. 2). The most immature cells of the plasma cell line, plasmablasts, are the first cells to elaborate immunoglobulins in measurable

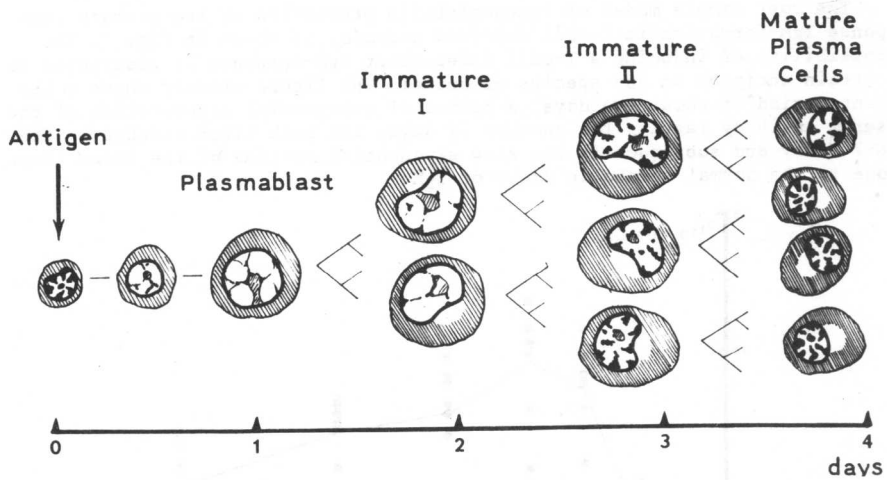

Fig. 2. Cell kinetics of plasmacell response: transformation (0-1 day) and clonal differentiation (1-4 day).

quantities: the quantity produced shows a sharp exponential rise during clonal development (see figs. 3, 7 and 5) first by the exponential growth, of the cell number per clone and secondly by the rapid growth of the immunoglobulin synthesizing apparatus per cell representing the maturation process and extending over some 7 mitoses. Anatomically the maturing plasmacells during clonal development move from the outer cortex towards the medulla where part of them is retained in the medullary strands as mature plasmacells. Another part is carried along with the passing lymph towards the blood stream and, presumably, sequestrated in the bone marrow.

(iii) Lastly the germinal centre response makes its appearance. Its first sign is the explosive appearance, 4 days after antigen administration, of large basophilic blast cells in the centre of lymphoid follicles (van Buchem, 1962) arising by transformation of small lymphoid cells. After its start as a blast cell response the germinal centres in some 24 hrs. acquire their characteristic micfoscopical aspect with large and medium-sized lymphoid cells showing many mitoses, and dispersed macrophages containing nuclear debris.

In contrast to the lymphoblastic T-cell response and the plasmacell response, which in their elementary form seem to consist of one more or less synchronous wave of cell transformation followed by clonal differentiation, the germinal centre reaction continues to be active for 2-3 weeks after a single antigenic stimulus. This continued activity appears to depend on the continued presence of antigen in the follicular centre and, as discussed further below, a continued supply of precursor B-cells. The end cells of germinal centre activity appear to be antibody forming cell precursors, part of which is distributed through the blood circulation to other peripheral lymphoid organs.

IgM-PRODUCTION

The most simple model of immunoglobulin production is the primary response IgM-formation in *T-cell deprived animals*, as shown in fig. 3. The possibility of inducing a T-cell independent IgM-response is restricted to certain antigens in any species of animal. The figure clearly shows a latent period of about two days, a period of exponential accumulation of the serum antibody lasting for another 2½ days, the peak titer reached by the 6-7th day and subsequently the slow exponential decline of the serum titer due to the normal metabolic decay of IgM.

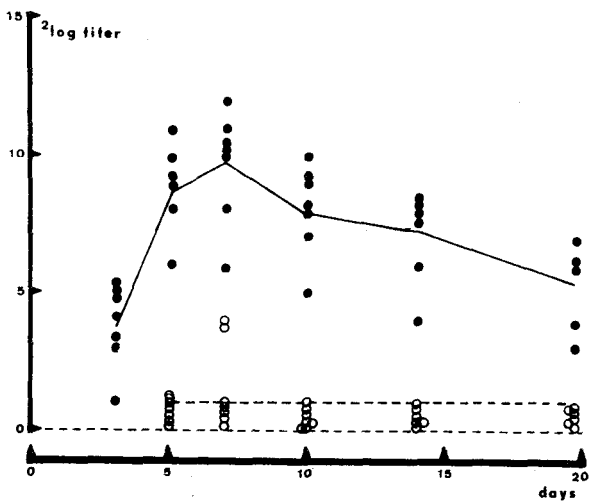

Fig. 3. Primary response to Salmonella java H-antigen in T-cell deprived (thymectomized and heavily irradiated) rabbits. Drawn line and black circles: anti-H serum titer. Dotted line and open circles: mercaptoethanol resistent anti-H serum titer. Intravenous administration of antigen on day 0.

Obviously the abrupt fall of IgM-production after its exponential increase does not represent a feedback inhibition by IgG, as IgG is lacking in these animals. Moreover the fall of IgM-production was not advanced by passively introducing homologous hyperimmune antibody 1-12 hrs. after the antigen.

In *normal* animals (fig. 4) a similar course of IgM production can be distinguished in the primary response. Although the overall titer curve (drawn

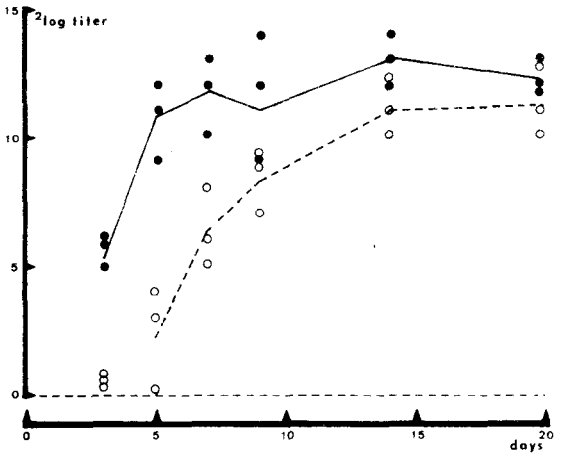

Fig. 4. Primary response to Salmonella java H-antigen in normal rabbits. Drawn line and black circles: anti-H serum titer. Dotted line and open circles: mercaptoethanol resistent anti-H serum titer. Intravenous administration of antigen on day 0.

line) in fact signifies IgM + IgG antibody, it can be hold to reliably represent IgM during the first days of the response, when the IgG titer (dotted line) is absent or low. There is a good correspondence between the IgM-response, the histological observations on the plasmacell response (fig. 2), and the data on direct (IgM) plaque forming cell counts.

When this first part of the response was investigated with varying antigen doses, the results shown in fig. 5 were observed. Below the antigen dosis needed to give a maximum response, a direct linear dose-response relationship is found between antigen dose and 6-7th day post-log phase peak titer. The lower antigen doses appear to proportionally lower the titer curve, without, however, changing its pattern.

A similar effect on the IgM titer curve -expressed in fig. 6 as a regression line of the (6th day) IgM peak titers- was obtained by irradiating rabbits with radiation doses varying from 0 through 500 rads before administering a standard (optimal) dose of antigen. Histologically, within this dosage range roughly proportional numbers of B-cells, i.e. follicular lymphocytes and marginal zone lymphoid cells, were seen killed in interphase. Above 450 rads all of these cells were destroyed and IgM-responsiveness was completely abolished for appr. 7 days.

These findings together seem to warrant the conclusion that the course and level of the IgM-response in its elementary form depends on

- the available number of precursor cells responsive to the antigen used,
- the percentage of these precursor cells to which the antigen actually gets access,

- for each antigen-stimulated precursor cell a fixed pattern of transformation, clonal multiplication and functional maturation up to an end-point of functional activity of immunoglobulin elaboration.

This state of affairs would seem to imply that effective antigenic stimulation of IgM precursor cells, under these experimental conditions, is restricted to a few hours at most. This seems to be correlated with the restricted time of antigen localization in the precursor cell regions (marginal zone, outer cortex) as mentioned above and the histological observations on the plasma cell response.

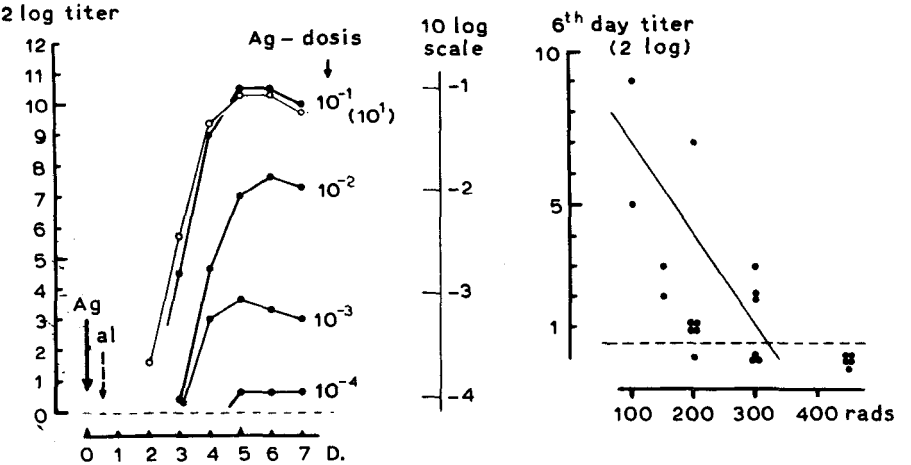

Fig. 5. (left) Effect of various doses Salmonella java vaccine (Ag-dosis) on serum anti-H agglutinin titer in normal rabbits. Each point: mean of three animals. Ag: intravenous antigen administration; al: administration of a small quantity of anti-H-hyperimmune serum.

Fig. 6. (right) Effect of various doses of total body irradiation, 24 hrs. before intravenous administration of Salmonella java vaccine, on 6th day anti-H serum titer in normal rabbits.

ORIGIN OF IgM-FORMING CELL PRECURSORS

As stated above, 450 or more rads of total body X-irradiation completely abolishes IgM responsiveness in rabbits for a period of appr. 7 days; histologically all B-cells are destroyed. When recovery from X-ray damage was investigated by giving a standard (optimal) dosis of antigen at moments between one day and three weeks after the irradiation under appropriate conditions, IgM production was first found upon antigen given on the 7th post-irradiation day. Within another 7-10 days IgM-forming potential had recovered to appr. its normal level as measured by the height of the (6-7th day) IgM peak titer (see fig. 11 further below). Obviously this signifies the reestablishment of a pool of precursor cells of about normal size. Histologically this correlated with the reappearance of large numbers of typical marginal zone lymphoid cells from the 7th post-irradiation day onwards.

In the rabbit the appendix and other gut-associated lymphoid tissue have been suggested by various investigators (Cooper et al., 1966) to represent *central* type of lymphoid tissue as they would autonomically, i.e. without antigenic stimulation, produce antibody forming cell precursors, and in this way represent an equivalent of the Bursa Fabricii of birds. Nieuwen-

huis (1971) studied the effect of various manipulations with the appendix on marginal zone lymphoid cell regeneration in the spleen (roughly graded from - to ++++) following 450 rads whole body X-irradiation. The results are shown in fig. 7. It is seen that *appendectomy* distinctly delayed the histological regeneration of marginal zone lymphoid cells; *appendix shielding* on the other hand distinctly improved and accelerated this recovery. Similar effects were observed on the recovery of IgM forming potential, expressed as the 6th day IgM (peak) titer upon antigenic stimulation 10 days after the irradiation. The most important point, however, was that *appendicostomy* with sterilisation of the appendiceal lumen by antibiotics, was as effective as appendectomy in delaying splenic marginal zone lym-

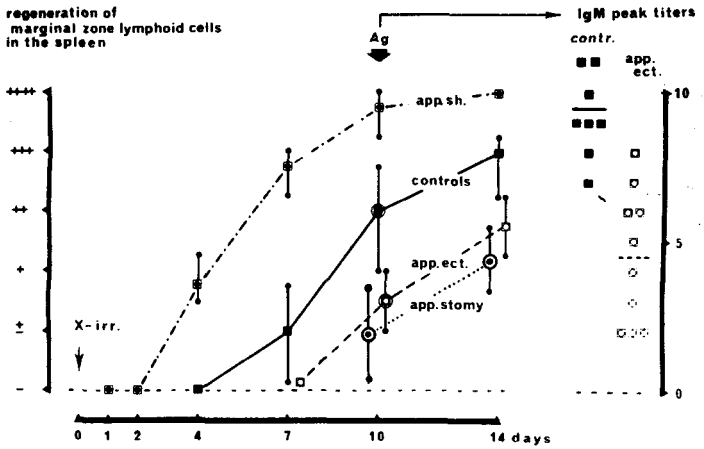

Fig. 7. Recovery of splenic marginal zone lymphoid cells (left ordinate) following 450 rads total body irradiation (X-irr.) in variously treated rabbits. App.sh.=irradiated with appendix shielded; App. ect.= irradiated following surgical removal of appendix; App. stomy = irradiated following appendicostomy with sterilization of appendix lumen.
Right: IgM peak (6th day) titers after antigen administration 10 days following X-irradiation in control (contr.) and previously appendectomized (app. ect.) rabbits. Redrawn after Nieuwenhuis (1971).

phoid cell regeneration. In the sterilized appendix regeneration of germinal centre activity was completely lacking; the other appendiceal lymphoid structures had regenerated normally.

The last result shows that appendiceal germinal centre activity, and with it production of any precursor cells by these germinal centres, is *antigen dependent*. It may in addition be noted that the receptor site specificities of the IgM-precursors do not seem to be related to the determinants of the intestinal antigens which presumably induced these germinal centres: whatever sign of secondary response type of immunoglobulin forming potential may be expected in case of related specificities was lacking in these experiments. Even low-affinity IgG precursor cells (see below) had not been formed.

Not only appendiceal but all germinal centres, throughout peripheral lymphoid tissue appear to generate immunoglobulin forming cell precursors. In labeling experiments end-cells of splenic germinal centre reactions showed up in part as marginal zone lymphoid cells of the splenic follicles

themselves; another part proved to be circulated towards follicular marginal zones in popliteal and axillary lymph nodes. Histological observations suggest a similar fate for the end-cells of lymph node germinal centres.

Further experiments both by Nieuwenhuis (ibid) and by Prop (1972) demonstrated that germinal centre activity itself always depended on the influx of directly bone marrow derived lymphoid cells. When germinal centre activity, induced in a lymph node by antigen administration, was abolished by local irradiation, the activity was resumed within four days when the bone marrow was shielded and the appendix irradiated, but not when the bone marrow was irradiated and the appendix shielded. In the latter case only some regeneration of marginal zone lymphoid cells was observed. Experiments of Prop (ibid) moreover showed that whole body X-irradiation with *shielding* of a lymph node in which active germinal centres had been induced resulted in fading-out of germinal centre activity.

Together these experiments led to the tentative concept (Nieuwenhuis, 1971), shown in fig. 8, that bone marrow derived B-cells, named B_1-cells, are first involved in an antigen-induced germinal centre reaction in which they are multiplicated and become precursor cells proper, the B_2-cells. These B_2-cells either make part of the marginal zone lymphoid cell popula-

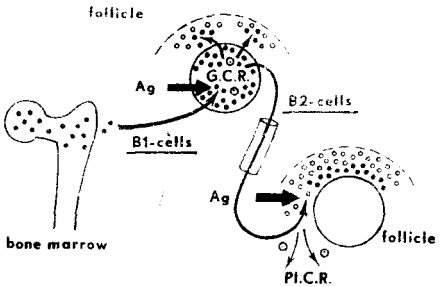

Fig. 8. Hypothetical fate of B-cells according to Nieuwenhuis (1971). B_1-cells involved in antigen-induced germinal centre responses (G.C.R.) give rise to B_2-cells settling either in marginal zone of follicle of origin or upon circulation in follicular marginal zones elsewhere. B_2-cells represent the precursor cells for plasmacell responses (Pl.C.R.).

tion of their follicle of origin, or upon circulation with the blood may settle in follicular marginal zones elsewhere in the lymphoid system. In these localizations appropriate antigenic stimulation may induce them to a plasma cell response (Pl.C.R.).

PRIMARY RESPONSE IgG PRODUCTION

IgG production during a primary antibody response shows a more complicated pattern. First of all IgG production against all antigens tested so far in mammals proved to be T-cell dependent. IgG responses in rabbits against Salmonella H-antigen have been studied by Mulder (1972). Normal primary response IgG formation in relation to IgM is shown in fig. 4. The early phase of this IgG response could be partly inhibited by the administration of a small quantity of anti-Salmonella hyperimmune serum 1-12 hrs. after the antigen; this results in a quite characteristic titer curve (fig. 9). A complete suppression of early IgG was obtained by local irradiation (700 rads) of the spleen 24 hrs. before intravenous administration of the

antigen (fig. 10); during this 24 hrs. period IgM responsiveness and late IgG forming potential had largely recovered.

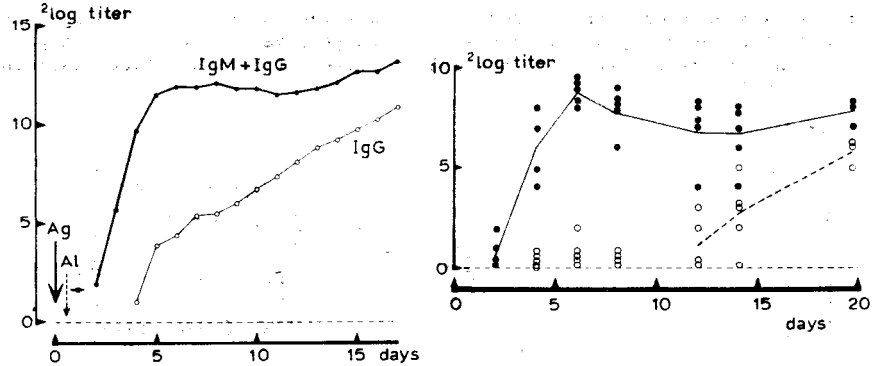

Fig. 9. (left) Effect of passive anti-H hyperimmune serum (al) on primary antibody response serum titer in rabbits (Ag = intravenous Salmonella java vaccine). Note two phases of IgG production. Compare with normal control (fig. 4).

Fig. 10. (right) Effect of local irradiation of the spleen on primary antibody response in rabbits to intravenously administered Salmonella java vaccine on (day 0). Suppression of early phase IgG production. Drawn line and black circles: anti-H serum titer. Dotted line and open circles: mercaptoethanol resistent anti-H serum titer.

When recovery from complete (IgM and IgG) unresponsiveness following 450 rads total body X-irradiation was studied (Mulder, 1972) it was found that IgM- and late IgG-forming potential (expressed as 7th day (peak) titer, and 14th day mercapto-ethanol resistent titer respectively) after antigen administered 7, 10, 16 or 23 days after the irradiation regenerated rapidly whereas early IgG-potential (8th day mercapto-ethanol resistent titer) was only restored very slowly (fig. 11). From these data the two phases of IgG production can be defined (best visualized in fig. 9) as an *early phase* in which the IgG titer first shows a rapid exponential rise and then reaches a maximum by the 8th day, and a *late phase* with a slow exponential rise for some 2-3 weeks. The differences between these two IgG production phases can be listed as follows:

Early phase IgG	Late phase IgG	
partly inhibited by passive antibody	not inhibited by passive antibody	fig. 9
suppressed by local irradiation 24 hrs. before antigen	not suppressed by local irradiation 24 hrs. before antigen	fig. 10
slow recovery (5-10wks) from suppression by 450 rads total body irradiation	rapid recovery (2wks) from suppression by 450 rads total body irradiation	fig. 11

These data together do not support the hypothesis of IgM producing cells switching over to IgG production; on the contrary the data tabulated suggest the existence of even two separate populations of specific IgG precursor cells for the early and late phase IgG respectively with different properties, a different origin and possibly a different cell kinetical pattern of antibody formation.

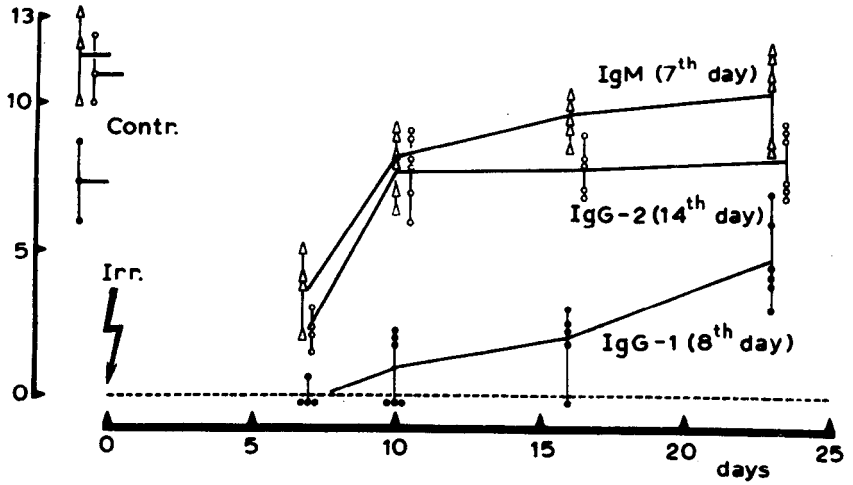

Fig. 11. Recovery of IgM, IgG-1 and IgG-2 responsiveness in rabbits after 450 rads total body irradiation (Irr). Slow recovery of IgG-1. All values are anti-H agglutinin serum titers: IgM = serum titer 7 days after Salmonella java vaccine (administered intravenously 7, 10, 16 or 23 days following irradiation); IgG-1 = mercaptoethanol resistent serum titer 8 d. after vaccination; IgG-2 = mercaptoethanol resistent serum titer 14 d. after vaccination. From data by Mulder (1972).

Early phase IgG precursor cells to any antigen preexist both in lymph nodes and the spleen of normal animals; late phase IgG precursor cells to a given antigen apparently do not, they seem to arise in the course of the immune response itself. This latter process is not severely affected by previous local irradiation of the lymphoid organ whereas such irradiation eliminates the preexistent early phase IgG precursors for many weeks.

The suppressibility of early phase IgG formation by passive antibody might result from accelerated immune elimination of antigen (though hard to accept in view of the later occurring late phase IgG) or it might represent competitive inhibition of low-affinity receptor sites of the early phase IgG precursor cells which would correspond to equally low-affinity IgG eventually produced by them. The late phase IgG precursors on the other hand seem to posses high-affinity receptor sites as these cells apparently can be induced even in the presence of autologous or passively introduced hyperimmune antibody.

The pattern of accumulation of early phase IgG in the serum (see fig. 9) suggests a cell kinetic pattern comparable to that of IgM, though delayed through T-cell dependency. The slow exponential rise of late phase IgG might be due to continuing growth of the responsible plasma cell clones, or to continued recruitment and induction of newly formed precursor cells.

SECONDARY RESPONSE IgG PRODUCTION

Though IgM formation does occur during secondary response, IgG production usually dominates.

Secondary response IgG production, like that of the primary response, is T-cell dependent: typical secondary responses are not observed in thymectomized (T-cell deprived) animals though the appropriate IgG-precursor cells are generated upon primary antigenic contact (Mulder, ibid). There is, however, a characteristic difference with the primary response. In T-cell dependent primary responses (IgM and IgG) a characteristic 1-2 days delay of plasmacell precursor transformation is always observed. It is easily read from titer curves (fig. 4 and 9) and clearly represented in lymph node histology. It is due to B-cell transformation depending on the T-cell lymphoblastic response having started. This delay is equally characteristically absent in secondary response (fig. 12). Cell transfer experiments (Mulder, ibid) have shown that this is due to the immediate availability of "primed" or "educated" T-cells, generated during the primary response.

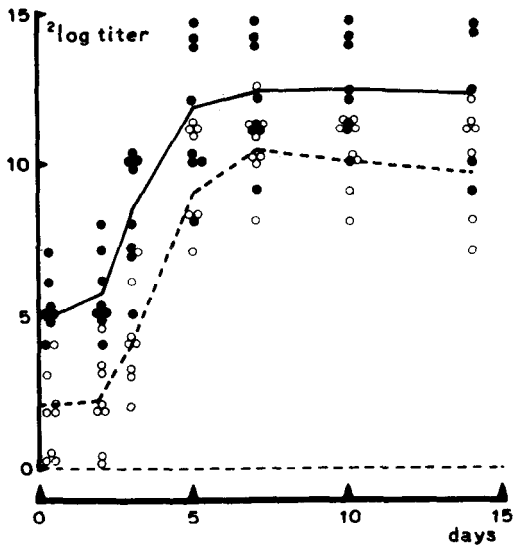

Fig. 12. Secondary anti-H agglutinin response. Serum titers following 2nd intravenous administration of Salmonella java vaccine to rabbits on day 0. Note absence of delay in IgG-response.
Drawn line and black circles: anti-H serum titer.
Dotted line and open circles: mercaptoethanol resistent anti-H serum titer.
Redrawn after Mulder (1972).

With regard to the cellular kinetics of the antibody forming cells in the secondary IgG response the combined observations of titer-curve (accumulation of antibody in blood serum), plaque forming cell counting and histology of the plasma cell responses, clearly show that the cellular kinetics follow a pattern which closely corresponds to that described for IgM during primary response. In lymph nodes this plasmablastic transformation is again seen in the outer cortex, particulary in the marginal zone regions between neighbouring follicles. There can be no doubt that the secondary response IgG- and IgM- precursor cells again are found among the population

of marginal zone lymphoid cells and follicular (mantle) lymphocytes. Like in primary response IgM formation the whole process of transformation and clonal differentiation of secondary response IgG forming cells has the character of one more or less synchronous wave. Like primary response IgM the IgG of the secondary response reaches a well defined peak titer, suggesting that the end-point of clonal development also marks the end-point of massive immunoglobulin elaboration. This is in accordance with plaque forming cell counts in which the number of indirect (IgG) plaque forming cells falls abruptly beyond the 4th day.

With regard to the affinity of secondary response antibody formation neither the IgG nor the IgM component are inhibited by earlier formed autologous or passively administered hyperimmune antibody. For the IgM it is not clear whether this really means an increase of affinity over primary response IgM as it is not certain whether the inhibition of primary response IgM responses by previously given hyperimmune antibody is due to competitive affinity between the hyperimmune antibody and the primary response IgM precursors or whether it is a non-competitive inhibition due to immune elimination of the antigen. Secondary response IgG has obviously higher affinity than the early phase IgG of the primary response. This situation would signify that the precursor cells for secondary response type IgG like those for late phase primary response IgG did not exist at the start of the primary response or in such small numbers as to escape observation but must have been formed during the primary response. So both with respect to the affinity and to their being produced during the primary response the precursor cells for secondary response IgG and late phase primary IgG response seem to have identical properties. This of course strongly suggests that they represent one kind of cells.

ORIGIN OF IgG-FORMING CELL PRECURSORS

Regarding the origin of the respective IgG precursor cell populations the very few available facts allow speculations only. The precursors for late phase IgG of primary response and for secondary response IgG, appear to arise during primary response. In accordance with earlier observations by Thorbecke and coworkers (1964) the most logical hypothesis would be that the germinal centre reactions of the primary response are responsible for specifically multiplicating these high-affinity IgG precursor cells.

With regard to the early phase IgG of low-affinity, the preexistence of its precursor cells and the very slow recovery of their pool both after local and after whole body irradiation suggest the possibility that these precursor cells have arisen in earlier germinal centre reactions. Their slow rate of recovery seems to suggest that they are not generated with random specificities like IgM precursor cells. The alternative would be that they are specifically produced in germinal centre reactions elicited by cross-reacting antigens and would consequently possess high affinity towards these latter antigens; cross-responsiveness of these cells to structurally related antigens presumably would be of low affinity.

As germinal centre reactions also make their appearance during secondary responses it seems logical to presume a new production of IgM precursors and of high affinity IgG precursors in secondary response germinal centres.