On the origin and fate of immunologically competent cells

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CHAPTER VII

Summary and conclusions

> Science is pure truth, do not let yourself be misled by facts.
>
> N.N.

In the *Introduction,* the apparent relation between the development of "immunity" and the lymphoid system is illustrated in a short historical review ending in the formulation of the concept of the immunologically competent cell "as a cell, which is fully qualified to undertake an immune response" (MEDAWAR, 1958).

In *Chapter I,* first the various views regarding the relations between lymphocytes and the lymphoid system, presented from ±1850 - ±1960, are discussed. From these observations three possible sources of lymphocytes emerged viz. germinal centers, the thymus and the bone marrow.

This period ended by GOWANS showing that the majority of the lymphocytes in the blood and the thoracic duct lymph is continuously recirculating from the blood to the lymph etc. by way of peripheral lymphoid tissues. Neither origin nor fate of these cells were known at that moment.

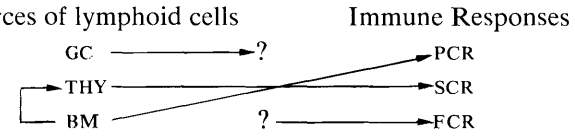
Next an evaluation of current views regarding the various types of immune responses is given viz. the plasmacell reaction (humoral immunity), the specific cellular reaction (cell mediated immunity) and the follicle center reaction (lymphocytopoiesis?, immunological memory?).

Regarding the relation between the various sources of lymphocytes viz. germinal centers (GC), the thymus (THY) and the bone marrow (BM) on the one hand, and the various types of immune responses viz. the plasmacell reaction (PCR), the specific cellular reaction (SCR) and the follicle center reaction (FCR) on the other, evidence is now available for the existence of: (1) a thymus-derived lymphoid cell system, comprising most of the lymphocytes present in the so-called "thymus dependent areas" (spleen: periarteriolar lymphocyte sheaths, lymph node: paracortical areas), and presumably involved in specific cellular reactions leading to cellular immunity (homograft rejection, delayed-type hypersensitivity), and (2) a non-thymus-derived lymphoid cell system comprising the lymphocte population of the follicular structures, and presumably related to the antibody forming capacity of an organism.

As regards the origin of the non-thymus-derived system the situation is rather conflicting as MILLER c.s. have clearly shown that antibody forming cell precursors are bone marrow derived, whereas GOOD and coworkers have postulated the gut-associated-lymphoid tissue to represent the mammalian equivalent of the avian bursa of Fabricius and consequently should be considered central lymphoid tissue controlling humoral immunity.

No conclusive evidence is available as yet regarding the origin of the precursor cell involved in the follicle center reaction, or the fate of the lymphoid cells presumably derived from germinal centers.

These considerations are summarized in the following diagram:



In the last section of this chapter the use of X-irradiation as a "dissecting" tool in immunology, and its effects on the lymphoid system and the immunological faculties of an organism are briefly reviewed.

In *Chapter II*, the objectives of the present study are explicitly formulated and a short outline of the experimental part of this study is given.

In *Chapter III*, materials and methods are described and discussed in detail. This holds especially for the various (local) ^{3}H-thymidine labelling techniques (p. 38) developed to study the cellular kinetics of the plasmacell reaction and the follicle center reaction, as well as the traffic within the lymphoid system of those lymphoid cells, which originate in the thymus, the bone marrow and the germinal centers.

In *Chapter IV*, an autoradiographic analysis is given of the plasmacellular reaction and early stages of the follicle center reaction in the spleen or the popliteal lymph nodes, following the i.v. or s.c. administration of an antigen respectively. From these studies it was concluded, that—upon antigenic stimulation—plasmablasts presumably develop from small or medium-sized lymphoid cells present among the marginal zone cells of the follicular structures in the spleen (figs. 1-4), or from small lymphoid cells present among the lymphocyte population of the experimentally (by local irradiation) induced primary nodules in the outer cortex of the popliteal lymph nodes (figs. 10-13).

Some 3-4 days after antigen administration *de novo* formation of ger-

minal centers was observed to start from small groups of medium-sized or blast-like cells, often found centered around a small blood vessel within a mass of dark staining lymphocytes (figs. 5, 6). The generating of small or medium-sized lymphoid cells within fully developed germinal centers (some 6-7 days after antigen administration) was clearly demonstrated (figs. 7-9).

In this way data were obtained regarding the cell, or cell types, as immunologically competent cell(s) involved in the plasmacell reaction. These data suggested this cell to be among the lymphocyte population of follicular structures.

In *Chapter V*, the thymic or bone marrow origin of lymphoid cells in peripheral lymphoid organs was studied.

Regarding the traffic of thymus-derived lymphoid cells to the so-called "thymus dependent areas" in the spleen and popliteal lymph nodes, it was shown that in the rabbit this population behaves identically to that described in some other laboratory animals (figs. 14-16). In this way a reference was obtained for bone marrow labelling experiments.

Using in vivo or in vitro ^{3}H-thymidine labelling of bone marrow cells no quantitatively significant relation between bone-marrow-derived lymphoid cells and follicular structures could be demonstrated (fig. 17).

In a series of experiments in which the regeneration of follicular structures in peripheral lymphoid tissues was studied following 450 rads whole body X-irradiation with or without the hind legs (bone marrow) shielded, again no indications were obtained for a bone marrow origin of the majority of the lymphocyte population of follicular structures. A striking observation, however, was the fact that regeneration of follicular structures in lymphoid tissues along the digestive tract, e.g. in the appendix, definitely preceded that in the spleen and popliteal lymph nodes.

In *Chapter VI*, the problem of the rabbit appendix being a central or peripheral lymphoid organ was studied in detail.

Simple light microscopical observations on follicular structures in the rabbit appendix suggested that these structures should be considered analogues to follicular structures in the spleen and popliteal lymph nodes (see text-figure VI-1, p. 78 and fig. 18).

Light microscopical observations on the neonatal development of the rabbit appendix disclosed the formation of primary nodules in special (dome shaped) areas during the very first days after birth (figs. 19, 20), and the subsequent development (from the 4th-7th day), within these primary nodules, of typical germinal centers, presumably preceding signs of plasmacell formation (figs. 21-23).

During these studies an as yet almost unknown type of lympho-epithelial relationship in the epithelium covering the dome shaped areas was noticed. Evidence is presented that this lympho-epithelial relationship developes around the time of birth by the infiltration of lymphoid cells into the epithelial lining of the future dome shaped areas (figs. 34-41). The invading lymphoid cells are engulfed within the cytoplasm of a special type of epithelial cells ("nurse cells") (fig. 39), so that in its mature form "clusters" of small or medium-sized lymphoid cells are found within the epithelium (fig. 26), each cluster being completely surrounded by the cytoplasm of its "nurse cell"(figs 27, 41). As to the fate of these cells indications were obtained for an emigration of these "cluster cells" into the appendiceal lumen(figs. 24 (arrow), 25, 26 (arrows), 30-33). The significance of this lympho-epithelial relationship as regards the postulated central role of the rabbit appendix is discussed.

Following local irradiation of the rabbit appendix, radiation damage to follicular structures within 24-48 hours was partially overcome by the influx of a population of small lymphoid cells at the site of the lymphocyte corona, which led to a complete repopulation of the dome shaped areas (figs. 42, 43). In the subepithelial zone, by the 3rd day after the irradiation, plasmablasts made their appearance amidst the marginal zone cells which in the mean time--by transformation—had arisen from the small lymphocytes of the lymphocyte corona (figs. 44, 45). From the 4th day onward *de novo* formation of germinal centers was observed also to derive from small lymphoid cells of the lymphocyte corona (fig. 46).

Following local ^{3}H-thymidine labelling of the rabbit appendix, a population of small or medium-sized lymphoid cells was found to develop, localizing in the central light staining part of its germinal centers (thinly populated area) (figs. 47, 48). In addition the migration of labelled small lymphoid cells from the appendix, presumably from its germinal centers, to follicular structures (lymphocyte corona and marginal zone) in the spleen was demonstrated (fig. 49, arrows). In experiments involving local ^{3}H-thymidine labelling of the appendix 18 hours after 450 rads whole body x-irradiation with the appendix shielded, the same phenomenon could be observed, and an enhanced regeneration of follicular structures in the spleen was noticed.

A contribution of the rabbit appendix to the post irradiation regeneration of follicular structures in the spleen, by the delivery of small lymphoid cells, was clearly demonstrated by either appendectomizing the animals prior to 450 rads whole body x-irradiation, or shielding the appendix during the irradiation (figs. 52-55). This contribution could be traced down to the germinal center compartment of follicular structures in the appendix by

subjecting the animals to appendicostomy with "sterilization" of the appendiceal lumen followed by 450 rads whole body x-irradiation (text-figure VI-3, p. 97 and figs. 50, 51).

A contribution of the rabbit appendix to the post irradiation restoration of the primary antibody forming potential became evident from experiments involving appendectomy or sham-appendectomy prior to 450 rads whole body x-irradiation and the i.v. administration of a paratyphoid vaccine on the 10th day post irradiation. In the appendectomized animals the 10th day post irradiation primary antibody forming potential was reduced to about 5% of sham-operated and irradiated controls (text-figures VI-2 (p. 95) and VI-4 (p. 98)). It was concluded, *"that, following 450 rads whole body x-irradiation the appendix contributes to the restoration of the primary antibody forming potential through the delivery of antibody-forming-cell precursors, these latter cells presumably being the folicle repleting class of lymphocytes derived from its germinal centers"* (p. 99).

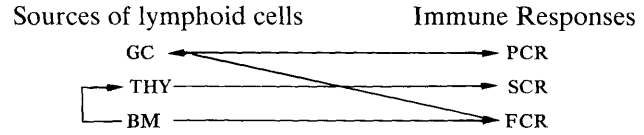
In the next series of experiments the investigations described in chapter IV regarding the cellular kinetics of germinal center activity in the spleen and popliteal lymph nodes, were extended. It was shown (1) that part of the population of lymphoid cells in the marginal zone is directly derived from the germinal center with which it is associated (figs. 56, 57a, 57, 59); (2) that there exists in the spleen and popliteal lymph nodes a population of lymphoid cells with migratory tendencies to follicular structures elsewhere (fig. 60); and (3) that upon antigenic (re-)stimulation presumably germinal-center-derived lymphoid cells (small lymphoid cells, marginal zone cells) become involved in a plasmacellular reaction (figs. 58, 61).

These latter observations in combination with the data obtained in the preceding section were interpreted as indicative of *the existence of a population of germinal center derived lymphoid cells, making part of the marginal zone cell system, with migratory tendencies to follicular structures elsewhere, and as antibody forming cell precursors involved in the plasmacell reaction.*

In the last series of experiments, animals first received a single dose of 450 rads whole body x-irradiation, and were then subjected to either a series of whole body x-irradiations (2 × 450 rads) with the appendix shielded or—inversely—to a series of local irradiations of the abdominal region (gut-associated lymphoid tissue) with the rest of the body (a.o. bone marrow) shielded (see figures 62-67). It was concluded that the *precursor cells involved in the formation of follicle center reactions (germinal centers) are bone marrow derived.* The significance of these conclusions regarding the observations by MILLER c.s. of the antibody forming cell precursors being bone marrow derived, and GOOD's hypothesis of the gut-

associated lymphoid tissue in the rabbit to represent the bursa equivalent is discussed in detail.

These considerations are summarized in text-figure VI-7 (p. 109), and are incorporated in the revised diagram below:



Finally, it was stated: "*To conclude we feel that a population of lymphoid cells, complementary to the T-cell system, has been identified, constituting the non-thymus derived part of the lymphoid system. This population of lymphoid cells, generally known as B-cells, is morphologically represented by all the follicular structures in the body. Essentially this population of lymphoid cells in follicular structures seems to be of dual origin: (1) directly bone marrow derived (B^1-cells) and involved in the formation of germinal centers, and (2) indirectly—through germinal centers—bone marrow derived (B^2-cells), represented by the marginal zone cell system, and as antibody forming cell precursors involved in the formation of plasmacells and thus humoral immunity. For both populations the lymphocyte corona should be considered the primary site of entry into follicular structures*" (p. 110).