The generation of germinal centers
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Germinal centers are clusters of B lymphoblastoid cells that develop after antigenic stimulation in follicles of peripheral lymphoid organs. These structures are thought to play a major role in the generation of B memory cells. This thesis is dealing with several aspects of these germinal centers. In the introductory chapter a survey is given of the literature concerning germinal centers. Moreover, in this chapter the findings of our studies as described in appendix papers I-VII, are included and discussed. The appendix papers are summarized below.

**Paper I** describes the production and characterization of three monoclonal antibodies (mAb) directed to B lineage associated determinants of the rat. These mAb, designated HIS14, HIS22 and HIS24, recognize epitopes of molecules with an apparent molecular weight of 205 kD, 210 kD and 205 kD respectively, possibly the B cell form of the rat leucocyte common antigen. All three antigenic determinants appear already early during B lymphopoiesis in bone marrow: the vast majority of pre-B cells are HIS14+ and HIS24+, and up to one-third are HIS22+. In peripheral lymphoid organs HIS14 reacts with virtually all sIg+B cells, whereas HIS22 and HIS24 label subsets of B cells. In tissue sections of peripheral lymphoid organs HIS14 stains all B cell compartments, HIS22 predominantly reacts with the lymphocyte corona and HIS24 stains both lymphocyte corona and germinal center; splenic marginal zones are not labeled by either HIS22 or HIS24. Thus, in the rat germinal center, B cells have a unique phenotype as they are HIS14+ and HIS24+ but HIS22−, allowing e.g. their isolation for further studies.

In **paper II**, the presence of T cells in germinal centers of rat spleen is demonstrated by immunoperoxidase staining using a panel of mAb directed to T cell (sub-)populations. Analysis of the phenotype of the germinal center T cells reveals that virtually all of them belong to the T helper subset. In addition, most of these T cells also express a determinant recognized by mAb ER3, which in cell suspensions reacts with only about 5% of the T helper cells. It therefore appears that the majority of germinal center T cells belongs to a subpopulation of T helper cells, characterized in addition to the ER2 marker, by the expression of the ER3 determinant.
In paper III it has been investigated whether immune complexes, retained on the surface of follicular dendritic cells are involved in the initiation of a germinal center reaction, as postulated by others. Therefore, rats were lethally X-irradiated, a procedure which causes lymphocyte depletion and simultaneously abolishes the follicular immune complex trapping capacity. After reconstitution with thoracic duct lymphocytes (TDL) and subsequent stimulation with sheep red blood cells (SRBC), germinal centers could be observed as early as 4 days after stimulation. The -as a result of the X-irradiation- impaired follicular immune complex trapping of horseradish peroxidase (HRP)-anti-HRP immune complexes recovered, however, 2 days later (i.e. by day 6). From these experiments it is concluded that the trapping and retention of immune complexes on follicular dendritic cells does not play a major role in the induction of a germinal center reaction, but is more likely involved in later phases of it.

In paper IV it is estimated from how many precursor cells each newly generated germinal center may be derived. Lethally X-irradiated AO rats were reconstituted with mixtures of TDL of AO and (AOxBN)F₁ origin in various ratios. The AO cells were tolerant for AOxBN cells by using TDL from AO→AOxBN bone marrow chimeras. To induce germinal center formation in recipient spleens, animals were intravenously challenged with SRBC. Rats were killed 5 days after immunization. Spleens were immunohistochemically stained with a mAb directed to an epitope of MHC Class II (Ia) antigens expressed in BN rats (but not in AO rats), allowing specific detection of AOxBN B cells (including germinal center cells). In spleens of rats reconstituted with mixtures of AO and AOxBN cells, three types of germinal centers were found: i) germinal centers entirely composed of AO cells, ii) germinal centers entirely composed of AOxBN cells and iii) germinal centers containing a mixture of both. The relative frequencies of these germinal center types indicate that in this experimental design, germinal centers develop oligoclonally from 1-3 precursor cells on average, for each newly generated germinal center.

Paper V describes the morphology of the spleen of the red-eared turtle to illustrate the phylogeny of germinal center formation. The spleen of the reptile is composed of red and white pulp. The white pulp is constituted
by densely packed lymphocytes surrounding blood vessels and comprises two distinct areas: i) an area surrounding (central) arterioles: the so-called periarteriolar lymphocyte sheath, and ii) an area surrounding the terminal arterioles encircled by reticular tissue (ellipsoid): the so called perielipsoidal lymphocyte sheath. Thus, the morphology of the spleen of the turtle closely resembles that of the avian spleen. However, by contrast with birds, germinal centers are never found in the turtle spleen, not even after antigenic stimulation. Despite this absence of germinal centers immune complex trapping has been observed in the spleen of the turtle (see paper VI).

In paper VI the capacity to trap and to retain immune complexes by the spleen of the red-eared turtle is reported. Intravenously injected HRP-anti-HRP immune complexes could be found in the splenic white pulp up to 16 days after administration. Immune complexes were found in only one white pulp compartment, viz. the perielipsoidal lymphocyte sheath where they were associated with non-phagocytic cells with a dendritic character. Thus, although germinal centers are absent in the turtle spleen (paper V) these spleens do have an important feature of germinal centers as found in mammals: the trapping and retention of immune complexes. The immune complexes are, however, not trapped in the form of clusters like in germinal centers, but are trapped throughout the perielipsoidal lymphocyte sheath.

Paper VII deals with the ontogeny of germinal center formation in the rat. As early as 7 days after birth, antigenic (SRBC) stimulation of neonatal rats resulted in germinal center formation in the spleen as observed 7 days later. Immunization of 3 day old rats (with or without the addition of adult spleen cells) did not lead to germinal center formation. However, transfer of 3 day old spleen cells to lethally X-irradiated adult recipients upon antigenic stimulation gave rise to germinal center formation in recipient spleens. This indicates that 3 day old spleens contain all essential lymphoid elements needed for the generation of germinal centers. Immunization of neonatal rats with SRBC did not cause an earlier appearance of primary follicles nor of follicular dendritic cells: both appear to develop simultaneously by day 14 after birth. From these
data it is concluded that in neonatal rats during the first days after birth the splenic microenvironment is too immature to allow germinal center formation, possibly due to an absence of mature FDC.