B Cell Proliferation in Follicles, Germinal Centre Formation and the Site of Neoplastic Transformation in Burkitt's Lymphoma

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INTRODUCTION

Both the nature of germinal centres and the origin of Burkitt lymphoma have given rise to an exceptional level of divergent speculation. The histological similarities between this tumor and germinal centres was recognised as long ago as 1961 by O'Conor. Mann et al (1976) noted that several American patients with Burkitt lymphoma had tumors which appeared to arise in germinal centres in lymph nodes or Peyer's patches. In a careful review of the histopathological evidence for this relationship Lennert (1978) concluded that on morphological grounds the Burkitt lymphoma cell most closely resembled an intermediate between a true centroblast and a B blast cell. This paper reports the findings of a study of synchronised germinal centre formation in rats. It shows that germinal centre formation is most apparent in primary B cell responses; secondary responses being associated with massive extrafollicular B cell responses. Finally the phenotype of germinal centre cells is compared with that of cells derived from Burkitt lymphoma. It is concluded that there is an extraordinary concordance between the cells found in normal germinal centres and the neoplastic cells of Burkitt lymphoma.

METHODS

Cell Lines

The following cell lines derived from patients with Burkitt lymphoma were studied BL2 (Derived by Dr G Lenoir, International Agency for Research on Cancer, Lyon); Daudi (Klein et al 1968); EB2 (Epstein et al 1965); EB4 (Epstein et al 1966); Kieti, Obagi and WW2BL (kindly provided by Professor A B Rickinson, the Department of Cancer Studies, Birmingham; Namalwa (Klein et al 1972); Raji and Jijoye (Pulvertaft 1965); Y4 (Lowe et al 1985).

Monoclonal Antibodies

The following monoclonal antibodies used in this study were included in the Third International Workshop on Leukocyte Typing (Ling et al 1987) BU-16 (CD9), J5 (CD10), BU-12 (CD19), L27 (CD20), BL-13 (CD21), MHM6 (CD23), BA-1 (CD24), WR-17 (CD37), KiB3, NU-B1 and KB61. OKT10 (CD38) was derived from the US type culture collection. AC2 (CD39) was kindly provided by Dr M Rowe (Rowe et al 1982).

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HIS 14 an antibody which identifies an antigen found on all B cells and HIS 22 which binds to an antigen found on follicular mantle B cells but not marginal zone or germinal centre B cells (Kroese et al 1985) were a kind gift from Dr Kroese and Professor Nieuwenhuis.

Quantitative Analysis of Antigen Expression on Cell Lines

This was carried out using an indirect immunofluorescence technique with a FACS IV (Becton Dickinson). The results were expressed as the 'mean gating value' after correction for background, which gives an approximate measure of antibody bound and hence amount of surface antigen per cell (Ling et al 1987).

Animals

Wistar rats were used which were bred under clean conditions in the animal house of the Department of Immunology, University of Birmingham. These animals were used because of the very low background level of germinal centres in their spleens.

Antigens and Immunization Procedures, Immunohistological Techniques and Quantitative Microscopy Methods

These are described in detail in Liu et al (1988) for the rat studies and by Hoffman-Fezer et al (1976) for human lymph nodes, spleens and tonsils.

RESULTS

Synchronised Germinal Centre Reaction

Experimental design: Conditions were found in which synchronised germinal centre formation could be studied in Wistar rats. In order to do this T cell help was provided by priming rats by intraperitoneal injection of alum-precipitated spider crab hemocyanin (MSH) mixed with $5 \times 10^9$ chemically killed Bordetella pertussis organisms. One month later the primary B cell response to two haptens 2,4-dinitrophenyl (DNP) and phenyloxazalone (OX) was studied following simultaneous i.v. injection with $50 \mu$g DNP-MSH and $50 \mu$g OX-MSH. The reason for using a two-hapten system was to allow simultaneous identification of OX-binding cells and DNP-binding cells in tissue sections by immuno-enzymatic methods using OX-horse radish peroxidase and DNP-alkaline phosphatase (Liu et al 1988). This allowed endogenously-produced hapten-specific surface immunoglobulin to be distinguished with confidence from passively bound antibody (Fig. 3b). The cell cycle status of cells during the response was determined either by injecting 5 mg Bromodeoxyuridine (BrdUrd) 2 hours before spleens were taken for analysis or by administration of BrdUrd at 0.8 mg/ml in rats' drinking water for 48 hrs prior to sacrifice. Cells which had taken up the label were identified by immunohistology (Liu et al 1988).