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PRODUCTION AND ANALYSIS OF A MONOCLONAL ANTIBODY REACTIVE TO THE MEMBRANE SURFACE OF CANINE LYMPHOCYTES

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A monoclonal antibody was produced from the spleen cells of a mouse immunized with lymphocytes which mainly contained large granular lymphocytes. This fraction was obtained by discontinuous Percoll density-gradient centrifugation. For the purpose of characterization of this hybridoma antibody, termed 177–1, reactivities to several cell populations were analyzed by flowcytometry. Moreover, immunoperoxidase stains were used for morphological analysis and distribution of 177–1–reactive cells.

1) The 177–1 had an IgGl subclass, and moleculer weights of target antigens against 177–1 were 68k and 74k, as estimated by the Western blotting technique.

2) It reacted with 8%–10% of peripheral lymphocytes under indirect immunofluorescence observation. It reacted poorly with nylon-wool-nonadherent-cells, plastic-dish-nonadherent-cells and thymocytes, which showed high reactivity with Thy–1 monoclonal antibody, but reacted highly with nylon-wool-adherent-cells, plastic-dish-adherent-lymphocytes, lymph node cells and spleen cells.

3) Lymphocytes stimulated with PWM showed high reactivity with 177–1 as compared to ConA-stimulated lymphocytes and nonstimulated lymphocytes.

4) The 177–1–positive cells in peripheral lymphocytes were small and medium in size. Monocytes and granulocytes had no reactivity to 177–1.

5) Anti 177–1 antigen was detected by histological observation on the cells in the germinal center of the lymph node and the spleen. The positive cells were some of the small and medium lymphocytes and the distribution corresponded to the SIg-positive region. In thymus, almost no 177–1–positive cells were detected.

These results are compatible with the view that monoclonal antibody 177–1 recognized a part of the antigen that is expressed on the surface of B lymphocytes. Further study is needed to clarify the role of anti–177–1 antigen and its function in B–cell differentiation.