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Anti-Brain Antisera Bind Primarily to Non-T Cells in Mouse Bone Marrow[1] (39962)

OLIVER W. PRESS,* CORNELIUS ROSSE,* AND JAMES CLAGETT†

*Department of Biological Structure and †Departments of Periodontics and Microbiology/Immunology, University of Washington, School of Medicine, Seattle, Washington 98195

The ability to discriminate accurately between functionally discrete subpopulations of lymphocytes on the basis of specific surface antigenic determinants has permitted a rapid expansion of our understanding of the immune system. Bone marrow-derived (B) lymphocytes are commonly identified by virtue of their readily detectable surface immunoglobulin (1), whereas thymus-derived (T) cells are usually delineated in cytotoxic or immunofluorescent assays utilizing either alloantisera such as anti-Thy 1 (or anti-θ) (1) or heteroantisera raised against thymocytes (2) or brain (3–5). Anti-brain heteroantisera have several advantages over other T-cell reagents. Because T-cell surface antigens are present in curiously high densities on brain tissue (3), large quantities of these potent T-cell reagents can be readily raised. However, the suitability of employing such antisera to identify T cells in lymphomyeloid organs is questionable since numerous reports have demonstrated reactivity of anti-brain sera with hemopoietic cells other than T lymphocytes (6–10). Furthermore, experiments in our laboratory have suggested that many murine bone marrow cells binding anti-brain antibodies are not T cells (11). In the present communication we have tested the validity of utilizing a goat anti-rat brain antiserum to identify T cells in the thymus, lymph nodes, spleen, and bone marrow of the mouse.

Materials and methods. Animals. Ten-week-old female CBA/J mice were obtained from Jackson Laboratories, Bar Harbor, Maine. Ten-week-old athymic "nude" mice were obtained from Dr. Karl Hellstrom, Department of Pathology, University of Washington. Inbred Lewis rats were obtained from Simonson Laboratories, Gilroy, California.

Antiserum preparation. Goat anti-rat brain antiserum was prepared according to Golub (3) by injecting goats with brains of Lewis rats emulsified in Freund's complete adjuvant as previously described (4, 5). The resultant antiserum is known to react with T cells of both the rat and the mouse (4, 5). The crude antiserum was purified by precipitation with saturated ammonium sulfate at 0°, and the 7s fraction was obtained by DEAE-cellulose chromatography. The antiserum was absorbed with mouse erythrocytes and mouse kidney. $F(ab')_2$ fragments of the antiserum were prepared by pepsin digestion prior to fluoresceination (11). Aliquots of the fluoresceinated anti-rat brain (FITC-ARB) reagent were absorbed ×3 with 0.5 packed volume of CBA/J thymocytes or nude mouse (CBA nu/nu) bone marrow.

Cell suspensions. Mice were killed by cervical dislocation and single-cell suspensions of thymi (Thy), mesenteric lymph nodes (LN), spleens (Spl), and bone marrow (BM) were prepared as previously described (13). A lymphocyte-enriched (82.1 ± 4.8% lymphoid cells) fraction (BML) of the marrow was obtained by sucrose gradient sedimentation (13).

Immunofluorescence. One million cells from each tissue were suspended in 50 µl of RPMI-1640 (Microbiological Associates) and incubated with 25 µl of FITC-ARB (or with one of the Thy-absorbed or nude BM-absorbed aliquots) at 0° for 45 min in the presence of 0.1% NaN_3. The cells were then washed ×3 with 3 ml of RPMI prior to preparation of wet mounts and examination in a Zeiss fluorescence microscope with a Kp500 exciter filter and a 530 barrier

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filter. Cell counts were performed by scoring 500–1000 consecutive cells for fluorescence from each tissue.

Results. The percentages of cells from each of the lymphomyeloid organs which stained with FITC-ARB are summarized in Table I. The percentages of stained cells observed in Thy (>99%), LN (68%), and Spl (46%) correlate well with the widely accepted T-cell constitutions of these organs (1). However, the percentage of FITC-ARB-positive cells observed in unfractionated CBA marrow (13%) is in excess of the recognized proportion of T cells in mouse BM [reviewed in (12)], particularly in view of the fact that lymphocytes comprise only 20–30% of whole marrow. The BML fraction of the marrow was enriched roughly threefold for lymphocytes (82.1 ± 4.8%) over whole BM, but contained a lower percentage of FITC-ARB-positive cells (10%). Furthermore, BM from nude mice possessed as many stained cells as normal CBA BM, despite the fact that nude mice possess very few mature T cells. Extensive absorption of FITC-ARB with CBA thymocytes abrogated the antiserum's staining of Thy and LN cells completely (Table I). Similarly, only 2% of Spl cells fluoresced when exposed to the thymocyte-absorbed FITC-ARB. However, staining of BM (13%) and BML (10%) was undiminished by removing the reagent's T-cell specificities (Table I). Repeated absorptions of FITC-ARB with nude BM eliminated the heteroantiserum's capacity to stain marrow cells (<1%), and confirmed the suspicion that the unabsorbed reagent was primarily staining hemopoietic cells unrelated to T lymphocytes in the bone marrow of CBA mice.

Discussion. These experiments demonstrate that anti-rat brain antisera can be used without reservation to identify T cells in mouse Thy and LN. This reagent can also be used confidently with Spl suspensions since 95% of FITC-ARB-positive cells from this tissue are T lymphocytes. However, absorption studies have shown that most cells in the BM which react with the anti-brain serum are not T cells. The exact identities of the non-T cells in the BML and Spl which stain with this heteroantiserum remain uncertain. Anti-brain sera have been reported by other workers to have specificities for hemopoietic stem cells (6), precursor T cells (8, 10), erythroid cells (7), and B cells (9). All these cell types (especially the first three categories) are much more prevalent in the BML and Spl than in LN and Thy (12, 14).

TABLE I. IMMUNOFLUORESCENCE WITH FITC-ARB.

	Unabsorbed (%)	Absorbed with thymocytes (%)	Absorbed with nude BM (%)
Thy	>99	<1	N.D.[a]
LN	68	<1	N.D.
Spl	46	2	N.D.
BM	13	13	<1
BML	10	10	<1
Nude BM	12	N.D.	N.D.

[a] Not done.

In conclusion, anti-rat brain antiserum has been shown to be a useful reagent for the identification of T cells in the Thy, LN, and Spl of the mouse. The ability to conveniently obtain this T-cell reagent in large quantities of uniform potency makes ARB especially useful for experiments requiring purification and conjugation of the antiserum to fluorescein or ferritin. Allo-anti-Thy 1 antisera are much more cumbersome to raise, titer, purify, and fluoresceinate in amounts large enough to allow utilization of the same batch for numerous experiments. However, certain additional absorption steps must be performed before anti-brain antisera can be used to identify T cells in the BM. The ARB may first be absorbed with nude BM to remove cross-reacting hemopoietic specificities to yield a T cell-specific reagent. Alternatively, one can determine the difference in staining indexes obtained using unabsorbed FITC-ARB as compared with thymocyte-absorbed FITC-ARB (15).

Summary. Anti-brain heteroantisera are widely employed for the delineation of T lymphocytes in lymphomyeloid organs despite compelling evidence demonstrating the cross reactivity of such reagents with unrelated hemopoietic cells. The experiments described in this communication utilized absorptions of a fluoresceinated goat anti-rat brain antiserum with thymocytes or nude bone marrow cells to determine the

validity of using such an antiserum to identify T cells in the thymus, lymph nodes, spleen, and bone marrow of CBA/J mice. Thymocyte absorption of this fluoresceinated antiserum removed virtually all of the staining of thymus, lymph node, and spleen cells by this reagent. In contrast, the percentage of labeled marrow cells was unchanged by thymocyte absorption of the anti-rat brain antiserum, although the staining could be eliminated by absorption with nude bone marrow. These results suggest that anti-brain antisera can be employed as dependable T-cell probes with suspensions of the thymus or peripheral lymphoid organs. However, the vast majority of marrow cells staining with the anti-brain antisera are not T cells. Therefore, anti-brain antisera should not be utilized to discern T lymphocytes in this tissue unless additional absorptions (with nude marrow) or special controls are performed.

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