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Cross-Reactivity between Human Thymus and Mouse Lymphoid Tissues, as Revealed by Rabbit Antiserum Against Human Brain[1] (38702)

GINO DORIA AND CARLO D. BARONI
(Introduced by R. A. Good)

CNEN-EURATOM Immunogenetics Group, Laboratory of Animal Radiobiology, C.S.N. Casaccia, (00100 Rome); and Institute of Pathological Anatomy II, Experimental Pathology Unit, University of Rome, 00161 Roma, Italy

All mice possess one or the other of two thymic antigens, θ-AKR and θ-C3H, which are present in high concentration only in thymus and nervous tissues (1, 2). Alloantisera to these antigens have been used to determine the proportion of thymus-dependent (T) lymphocytes in lymphoid tissues (3), and to facilitate the understanding of the functions of T and thymus-independent (B) lymphocytes in the immune response both *in vivo* (4) and *in vitro* (5, 6).

The advantage of producing large amounts of antiserum and the interest in extending these studies to other species including man have created the need of xenogenic antisera raised by immunization across species. Thus, using mouse antisera Potworowski and Nairn (7) found in rats a species specific thymic antigen which appears to be absent from brain. Asakuma and Reif (8) hyperimmunized rabbits with mouse brain and obtained cytotoxic antibodies specific for mouse thymocytes. After absorption with C3H tissues rabbit antisera to AKR brain could distinguish between θ-AKR and θ-C3H thymocytes (8). Golub (9) immunized rabbits with mouse brain and obtained an antiserum with anti-θ-like cytotoxic activity. When tested with mouse cells, this antiserum was toxic for thymus but not marrow cells, inhibited the graft-versus-host reaction and the *in vitro* primary response of spleen cells to sheep RBC; however it did not influence B cell-derived anti-body-forming cells (9). Golub also found that the brain tissue of several species (mouse, guinea pig, rat, horse, cat, pig, man, and rabbit) can absorb out the anti-mouse thymocyte activity of goat anti-mouse brain antiserum, and that rabbit anti-rat, -human, or -dog brain antiserum contains cytotoxic antibodies specific for mouse thymocytes (10).

In man, T cells have thus far been distinguished from B cells mainly on the basis of their ability to form rosettes with sheep RBC and of the lack of demonstrable Ig and receptors for C′3 (11). The results obtained using xenogenic antisera and tissues of several species have suggested the feasibility of devising tools for the identification of a human θ-antigen. With this aim, rabbits were immunized with human brain. Before being tested with human lymphoid cells, the antisera were monitored for the presence of antibodies cross-reacting with mouse tissues. The results of these cross-reactions are reported here.

Materials and Methods. Preparation of rabbit antisera. Rabbit anti-human brain antisera were obtained from rabbits immunized with human brain. The brain prepared for each injection was a piece of parietal lobe, freed of meningeal blood vessels, taken from an adult human cadaver 24 hr after death. The brain tissue was mechanically dissociated, homogenized in a Potter grinding vessel with phosphate buffered saline (PBS) and washed twice with PBS (1500 rpm for 10 min). The packed brain tissue was then emulsified with an equal volume of Freund's complete adjuvant (Difco). One ml of emulsion was injected subcutaneously into four rabbits at weekly intervals. One week after the third injection the animals were bled from the heart. After 1–5 mo, the rabbits were reinjected as above and bled again one week later. During this period the majority of the animals died with symptoms of encephalomyelitis. Sera were heated at 56° for 30 min and then stored at −20°.

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Absorption was carried out by incubating equal volumes of serum and of a given tissue (dissociated mechanically, washed with PBS and then packed by centrifugation) at 4° for 18 hr. The mixture was then centrifuged at 1500 rpm for 15 min and the supernatant recentrifuged at 10,000 rpm for 15 min to discard cell debris. Residual antibody titer, if any, was not affected by a second absorption with the same tissue.

Sera from different bleedings and rabbits were never pooled.

Cytotoxic test. Antisera were tested against nucleated cells from mouse thymus, mesenteric lymph node, or spleen by the method of ^{51}Cr release (12). The technique was essentially that used by Raff and Wortis (3) to detect θ-antigen on the surface of lymphocytes. Tissues were removed from 6-wk old C3HeB/FeJ ♂ mice and teased apart in Eagle's medium containing 0.1% BSA (Armour). Cells were washed three to four times (1500 rpm for 10 min) with Tris–buffered NH_4Cl to lyse red blood cells. The nucleated cell concentrations were adjusted to 2×10^7/ml and then the suspensions were mixed with $Na_2Cr^{51}O_4$ at a final concentration of $\sim 50/\mu Ci/ml$ and incubated at 37° for 45 min. Thereafter, cells were washed once with Eagle's medium, then six times with veronal buffered saline containing 0.1% BSA (VBS), filtered through 20 μnylon mesh, and adjusted to 1×10^6 cells/ml in VBS. One-tenth of ml of the labelled cell suspensions was incubated with an equal volume of antiserum (AS) or human brain-absorbed normal serum (NS), both undiluted and diluted with VBS in a twofold manner, at room temperature for 15 min. Thereafter, 0.1 ml of agar-absorbed (13) guinea pig complement (Sclavo) was added (final complement dilution, 1:10 in VBS) and the mixture incubated at 37° for 30 min. The reaction was stopped by addition of 1 ml of cold PBS. The tubes were centrifuged at 1500 rpm for 10 min, and the supernatants decanted and counted in a well-type scintillation counter. Duplicate 0.1 ml aliquots of each cell suspension were incubated as above with 1.2 ml of Triton, instead of serum, complement, and PBS, to produce complete lysis and Cr^{51} release. From the radioactive counts released in supernatants, the following cytotoxic index was determined: $AS - NS/Triton - NS \times 100$. When target mouse lymphoid cells were incubated with NS and complement the Cr^{51} release was within 10% of the triton control.

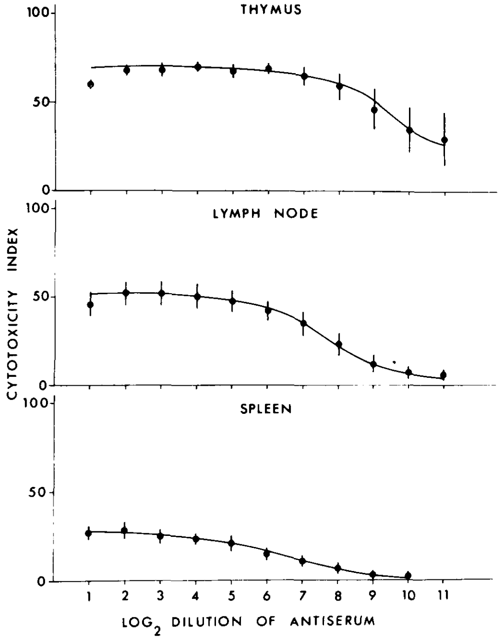

FIG. 1. Cytotoxicity index as a function of log$_2$ final dilution of rabbit anti-human brain antiserum for C3HeB/FeJ ♂ mouse thymus, lymph node, and spleen cells. Points represent the means of seven (thymus), five (lymph node), and six (spleen) experiments; vertical bars, two standard errors. In each set of experiments different antisera were repeatedly

Results and Discussion. Several rabbit anti-human brain antisera (different bleedings from different animals) were repeatedly tested for cytotoxicity against mouse thymus (seven experiments), mesenteric lymph node (five experiments), and spleen (six experiments) nucleated cells. Antisera from the first and second bleeding of all rabbits had similar cytotoxic titers. For each tissue, the results were pooled and the mean cytotoxic index is presented in Fig. 1 as a function of the log$_2$ final dilution of the antiserum in the reaction mixture before addition of complement. Plateau mean values were higher for thymus (70%) than for lymph node (50%) and spleen (25%) cells. These

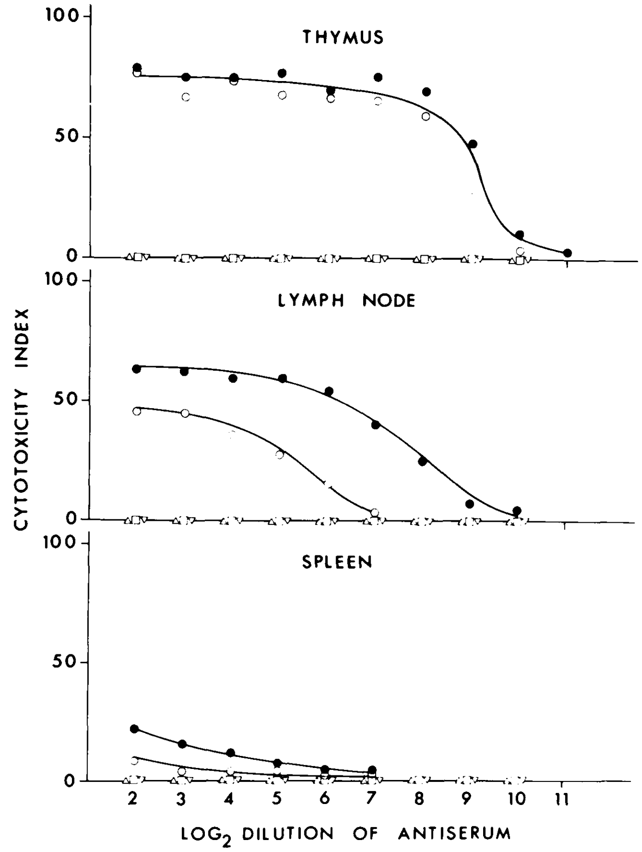

FIG. 2. Cytotoxic index as a function of $\log_2$ final dilution of a rabbit anti-human brain antiserum for C3HeB/FeJ ♂ mouse thymus, lymph node, and spleen cells. Effect of absorption of the antiserum with human thymus cells, O——O; human brain, □——□; mouse thymus cells, △——△; mouse brain, ▽——▽. Unabsorbed antiserum, O——O.

values and the shapes of the curves are similar to those obtained by Raff and Wortis (3) with AKR anti-θC3H antiserum, thus indicating that mouse cells crossreacting with human brain have a percent distribution similar to that of θ-positive cells. Furthermore, when rabbit anti-human brain antisera were absorbed with mouse brain or mouse thymus cells, cytotoxicity against mouse thymus, lymph node, and spleen cells was completely lost, as when the antisera were absorbed with human brain (Fig. 2). Using the Jerne technique, when 0.1 ml of rabbit anti-human brain antiserum was plated with 2.5 ml of agar, 0.1 ml of sheep RBC, and 0.1 ml of a suspension of spleen cells from mice immunized against sheep RBC,[2] as in a similar method previously described (14), no inhibition was observed of the development of direct PFC anti-sheep RBC. This result suggests that the rabbit antiserum at a final dilution of 1:28 did not contain antibodies against B cell-derived antibody forming cells. Unlike the absorption with mouse brain, however, ab-

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[2] Six C3HeB/FeJ ♂ mice were injected intraperitoneally with 1 ml 1% sheep RBC and sacrificed 4 days later. The spleens were pooled and teased apart, and cells suspended in 6 ml of Eagle's medium. After filtration through 20 μnylon mesh, aliquots of 0.1 ml of the spleen cell suspension were used in the Jerne assay.

sorption of an AKR anti-θ C3H antiserum[3] with human brain did not remove the cytotoxic activity against mouse thymus cells. Therefore, as human brain does not react with anti-θ antibodies, it seems unlikely that the mouse antigen cross-reacting with human brain could be the θ antigen.

Figure 2 also shows the results of one experiment in which a rabbit antihuman brain antiserum was used after absorption with human thymus cells (3).[4] Cytotoxicity against mouse thymus cells remained significantly unchanged, although the antiserum had been absorbed twice. However, some cytotoxicity was lost when the antiserum was tested against mouse lymph node and spleen cells. These findings were consistent in repeated experiments with different antisera. It may be postulated that human brain and mouse thymus cells share two antigens, *a* and *b*, of which only *a* is present in human thymus cells. In the mouse, as thymus lymphocytes migrate to peripheral lymphoid tissues there is a loss of either antigen. Accordingly, in lymph node and spleen some T cells possess only *a* while other T cells carry only *b* antigen. Thus, absorption with human thymus cells removed only antibodies anti-*a* leaving anti-*b* antibodies able to kill all mouse thymocytes and a proportion of T cells in mouse lymph node and spleen. Loss of antigens, such as TL, is known to occur in thymus-derived lymphocytes (15). Complete removal of cytotoxicity by absorption with mouse brain implies that also this tissue, like mouse thymus, possesses both *a* and *b* antigens.

Crossreactivity between human brain and mouse tissues other than the lymphatic ones was investigated by studying the effect of absorption with mouse liver[5] or erythrocytes[6] on the cytotoxicity of rabbit antihuman brain antiserum against mouse thymus, lymph node, and spleen cells. The results of two experiments with two different antisera, each of which tested separately, were pooled and are shown in Fig. 3. They indicate that: (a) neither liver nor RBC removed any citotoxicity when the absorbed antiserum was tested against thymocytes; (b) liver but not RBC could absorb to some extent cytotoxic antibodies against lymph node cells; (c) liver and RBC were both effective in absorbing to a greater extent cytotoxic antibodies against spleen cells.

TABLE I. TISSUE DISTRIBUTION OF CROSS-REACTIVE ANTIGENS.[a]

Tissues	Antigens a	 b	 c	 d
Human brain	+	+	+	+
Human thymus	+	−		
Mouse brain	+	+	+	+
Mouse thymus cells	+	+	+	+
Mouse lymph node cells	+	−	−	− or +
	−	+	−	− or +
	−	−	+	− or +
Mouse spleen cells	+	−	−	−
	−	+	−	−
	−	−	+	−
	−	−	−	+
Mouse liver			+	
Mouse erythrocytes				+

[a] Presence (+) or absence (−) of cell antigens; missing sign denotes lack of information. Each row represents a cell type specified by the presence of at least one antigen. Hence, while each mouse thymocyte possesses all four antigens, mouse lymph nodes and spleen are heterogenous tissues as they may contain as many as three- and four-cell types, respectively.

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[3] AKR anti-θC3H antiserum was prepared in AKR ♀ mice by four subcutaneous injections, each every 2 wk, of the following increasing numbers of nucleated thymocytes from C3HeB/FeJ ♀ mice: 1, 5, 25, and 100 × 10^6. One week after the last injection mice were bled and the antisera pooled.

[4] Thymus was obtained at autopsy from a human newborn 24 hr postmortem. Cells were prepared by mechanical dissociation, washed with PBS, and packed by centrifugation.

[5] C3HeB/FeJ ♂ mice were killed by decapitation. Livers were perfused by injection of 10 ml of PBS into the portal vein and, after removal of the gall bladder, homogenized, washed with PBS, and packed by centrifugation.

[6] Erythrocytes were prepared from heparinized blood collected from C3HeB/FeJ ♂ mice by heart punction, centrifuged, and washed with PBS four times, each time discarding the buffycoat.

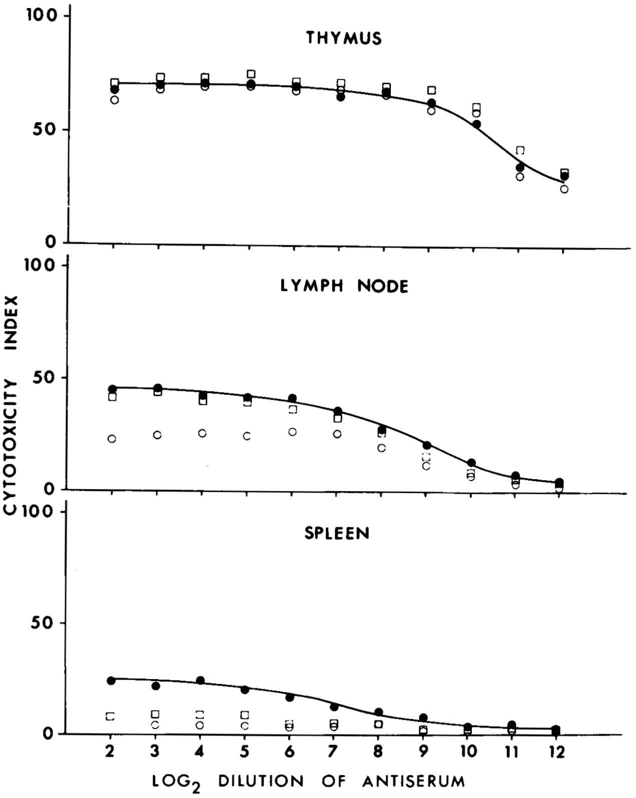

FIG. 3. Cytotoxic index as a function of $\log_2$ final dilution of a rabbit anti-human brain antiserum for C3HeB/FeJ ♂ mouse thymus, lymph node, and spleen cells. Effect of absorption of the antiserum with mouse liver, ○; mouse erythrocytes, □. Unabsorbed antiserum, ○——○.

These findings suggest that human brain possesses additional antigens, say *c* and *d*. Antigen *c* is shared by mouse liver, lymph node, and spleen cells. Antigen *d* is present on mouse erythrocytes and spleen cells but cannot be detected in lymph node cells. Since mouse thymus cells and brain can absorb all cytotoxic antibodies from rabbit anti-human brain antiserum (Fig. 2), it follows that antigen *c* and *d* are both present in each of these mouse tissues.

Based on results from all experiments in which mouse tissues were used as target or for absorption of rabbit antisera against human brain, Table I shows a tentative distribution of the four antigens *a*, *b*, *c*, and *d* in brain and lymphoid tissues of man and mouse. These antigens are all present in human and mouse brain and in mouse thymus, while human thymus possesses only one of the four antigens. In mouse thymus, antigens *a*, *b*, *c*, and *d* are all present on the same cell. As to mouse peripheral lymphoid tissues, the datare a compatible with the existence of as many as three-cell types in lymph nodes and four-cell types in spleen. The existence of more than one of these antigens on the same cell in peripheral lymphoid tissues should imply no reduction in cytotoxicity when the antiserum is absorbed with human thymus or mouse liver or erythrocytes and then tested against lymph node or spleen cells. In fact, reduction in cytotoxicity versus these cells was always observed when the antiserum had been absorbed with human thymus or mouse liver. The finding that cytotoxicity versus lymph node cells remained unchanged after absorption with mouse erythrocytes suggests that antigen *d* in lymph nodes, but not in spleen, either is absent or coexists with antigen *a* or *b* or *c* on a same cell.

Further experiments based on sequential

absorption should indicate whether some of the postulated antigens are identical. This may restrict to fewer antigens and cell types the cross-reactions found in the present study.

Summary. Rabbit anti-human brain antisera were found cytotoxic for mouse thymus, lymph node, and spleen cells. Absorption with human thymus cells removed some of the cytotoxic antibodies. Cross-reactions were also detected among human brain and thymus and mouse brain, liver, and erythrocytes.

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