

SURFACE IMMUNOGLOBULINS ON THYMUS AND THYMUS-DERIVED LYMPHOID CELLS*

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The initial interaction of antigen with lymphoid cells requires the presence of a specific surface antigen receptor. There is increasing evidence for the presence of such a receptor on both nonthymus-derived (B) lymphocytes¹ (1-3) and thymus-derived (T) lymphocytes (3, 4). A number of studies (4-9) suggest that this receptor may be immunoglobulin in nature. Surface immunoglobulins have been directly demonstrated on B cells (10-13) but such a direct demonstration on the surface of T cells has been lacking. This paper describes the presence of immunoglobulin light chains on the surface of thymus and thymus-derived lymphocytes.

Materials and Methods

Animals.—CBA and (CBA × C57BL)F₁ mice raised and maintained at the Hall Institute were used throughout. The mice were of either sex and were 50-120 days of age.

Cell Suspensions.—Thymuses were obtained from CBA mice of 50-70 days of age. Cell suspensions were prepared by passing the thymuses gently through a fine stainless steel mesh into cold Eisen's balanced salt solution (EBSS). Thoracic duct lymphocytes (TDL) from 120-day old CBA mice and H2-activated thymus-derived cells in the thoracic duct effluent (TTDL) were obtained by procedures described below. Cells were washed once in EBSS and four additional times through fetal calf serum (FCS) gradients according to the method of Byrt and Ada (14) to remove debris. The cells were finally suspended in 10% FCS in Dulbecco's solution and counted in a hemacytometer. The viability of thymus suspensions and TDL or TTDL suspensions were generally 90-95% and 98-99%, respectively.

Operative Procedures.—Thoracic duct fistulas were prepared using the modification of Miller and Mitchell (15) of the technique described by Boak and Woodruff (16). Generally the mice received an intravenous infusion of isotonic saline during the first 24 hr after cannulation to ensure optimal yield of lymphocytes. The cells were collected in 10% FCS in cold Dulbecco's

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¹ *Abbreviations used in this paper:* B, nonthymus-derived; EBSS, Eisen's balanced salt solution; FCS, fetal calf serum; PAPS, polyaminopolystyrene; RFC, rosette-forming cells; T, thymus-derived; TDL, thoracic duct lymphocytes; TTDL, H2-activated, thymus-derived thoracic duct lymphocytes.

solution. Preservative free heparin (Evans Medical Ltd., Liverpool, England) was used to prevent coagulation.

Preparation and Collection of TTDL.—The TTDL were obtained from thoracic duct cannulation of 120-day old (CBA × C57BL)F₁ male mice. These mice had undergone whole body irradiation (800 R) followed by intravenous administration of 100 × 10⁶ CBA thymus cells (50–56-day old female donors). The TTDL were collected starting 4 days after irradiation. The details of these procedures are described elsewhere (17).

Anti-Immunoglobulin Materials.—Antisera against mouse immunoglobulins were prepared in rabbits according to methods described previously by Herzenberg and Warner (18). The antisera with their corresponding immunogens were as follows: anti-light chain (κ) = anti HPC-2 (diethylaminoethyl [DEAE] fraction of Bence Jones kappa protein from BALB/c mouse bearing plasma cell tumor HPC-2); anti-IgM = anti-HPC-76 (starch gel electrophoresis fraction of serum from BALB/c mouse bearing plasma cell tumor HPC-76); anti-polyvalent heavy chain = anti-NZB normal serum globulin. The anti-polyheavy chain serum was shown to have high titers against μ , α , and γ 2 heavy chains.

The gamma globulin fraction of the rabbit antiserum was then separated by starch gel electrophoresis in Veronal buffer, pH 8.2. This globulin fraction (IgG) was then tested for anti-immunoglobulin activity by precipitation with ¹²⁵I-labeled purified myeloma proteins (18). In the case of the monospecific anti- μ serum, all absorptions to remove other antibody activities were made with immunoglobulins conjugated to polyaminopolystyrene (PAPS) (18), and the light chain activity of the anti-polyvalent heavy chain serum was removed by absorption with soluble purified HPC-4 (DEAE fraction of Bence Jones protein from [NZB × BALB/c]F₁ mouse bearing plasma cell tumor HPC-4).

In some cases where indicated, thymus absorptions of the anti-immunoglobulin protein were performed. This was done by two successive incubations for 1 hr at 0°C with thymus suspensions from young BALB/c or (CBA × C57BL)F₁ mice. (The equivalent of 5 thymuses/ml of protein solution containing 2–6 mg/ml)

Blocking of Anti-Immunoglobulin Protein.—The specific anti-immunoglobulin activity of the various rabbit globulin fractions was removed (blocked) by purified myelomas coupled to PAPS with the exception of the light chain (HPC-4) which was in solution. The absorptions were performed as follows: anti- κ absorbed with HPC-4 (κ) or HPC-1 (IgA- κ) and MPC-86 (IgG2b- κ); anti-polyheavy chain absorbed with HPC-1 (IgA- κ), MPC-25 (IgG1- κ), MPC-86 (IgG2b- κ), MOPC-104 (IgM- λ), GPC-7 (IgG2a- κ).

Iodination of Anti-Immunoglobulin Protein.—The globulin fractions of anti-immunoglobulin sera were iodinated with ¹²⁵I according to the method of Klinman and Taylor (19) with minor modifications in that 10% FCS in Dulbecco's solution was used to wash through the ion exchange column. The specific activity of the preparations varied between 4–6 μ Ci/ μ g protein.

Cell Labeling.—The cell suspensions were labeled with radioiodinated anti-immunoglobulins according to the method described by Bankhurst and Warner (10). 5 million viable cells were incubated for 1 hr at 0°C with labeled protein (2000–4000 ng in 0.25 ml total incubation volume) and subsequently washed four times through FCS gradients (14) before radioautography.

Radioautography.—The procedures for radioautography have been described by Byrt and Ada (14). The cells were stained with Giemsa, and only lymphoid-like cells of definitive morphology were scored. Positive cells had at least 15 grains on their surface or immediately adjacent to the surface. Clumped cells were excluded. 300 cells were counted on each slide to obtain the percentage of labeled cells.

RESULTS

Per Cent Labeling of Thymus, TDL, and TTDL Cell Suspensions.—TDL cell suspensions labeled on short exposure (4–6 days) with anti-light chain and

anti-heavy chain radioiodinated IgG (Table I). The percentage of labeled cells in the TDL preparation remained essentially unchanged between the short (4-7 days) and prolonged (30-60 days) exposure times (19.0 and 17.9% average, respectively, with anti-light chain) even though the number of grains per cell increased. The TDL labeling with the anti-polyheavy chain IgG (20.2% with prolonged exposure) was approximately equal to the anti-light chain labeling. Of interest is the experiment performed with anti- μ which showed a percentage of labeled cells (17.6% on prolonged exposure) approaching the percentage

TABLE I
Labeling of TDL, TTDL, and Thymus Cell Suspensions by Unblocked and Blocked Anti-Immunoglobulin IgG

| Experiment | Cell source | Percentage of labeled cells | | | | | | | | | | | |
|------------|-------------|-----------------------------|------|-------------------------|-----|------------------------|------|--------------------------------|-----|-------------|------|------|-----|
| | | Anti- κ * | | Anti- κ blocked* | | Anti-poly-heavy chain† | | Anti-poly-heavy chain blocked† | | Anti- μ | | NRG§ | |
| | | S | L | S | L | S | L | S | L | S | L | S | L |
| 1 | TDL | 18.6 | 16.6 | 0.3 | — | 20.0 | 32.0 | 0 | 4.0 | | | | |
| | TTDL | 0.6 | 50.0 | 0 | 0 | 0 | 1.6 | 0 | 0 | | | | |
| | Thymus | 2.6 | 14.3 | 0.3 | 3.0 | 0 | 1.3 | 0 | 0.3 | | | | |
| 2 | TDL | 15.3 | 17.0 | 0 | 0 | — | 11.3 | 0.3 | 3.0 | | | | |
| | TTDL | 1.3 | 42.0 | 0.3 | 1.6 | 0 | 0 | 0 | 0.3 | | | | |
| | Thymus | 1.6 | 19.0 | 0.3 | 2.0 | 0 | 0 | 0 | 0.3 | | | | |
| 3 | TDL | 23.0 | 20.0 | 0 | 1.0 | 17.0 | 17.3 | 0 | 1.0 | 12.0 | 17.6 | 0 | 0 |
| | TTDL | 0 | 19.0 | 0 | 2.6 | 0 | 0.3 | 0 | 0 | 0 | 0 | 0 | 0.3 |
| | Thymus | 1.0 | 10.0 | 0 | 2.6 | 0.1 | 1.0 | 0 | 0 | 0 | 0.3 | 0 | 0 |
| Average | TDL | 19.0 | 17.9 | 0.1 | 0.5 | 18.5 | 20.2 | 0.1 | 2.7 | | | | |
| | TTDL | 0.6 | 37.0 | 0.1 | 1.4 | 0 | 0.6 | 0 | 0.1 | | | | |
| | Thymus | 1.7 | 14.4 | 0.2 | 2.5 | 0.03 | 0.8 | 0 | 0.2 | | | | |

* These proteins in experiments 1 and 2 were thymus absorbed. The proteins in experiment 3 were not.

† These proteins in 1 and 3 were thymus absorbed.

§ Normal rabbit globulin.

|| S = 4-7 day exposure; L = 30-60 day exposure.

labeled with anti-polyheavy chain IgG. In all cases binding activity on the TDL, TTDL, or thymus could be selectively removed by preabsorption of the anti-immunoglobulin IgG with purified myeloma. On the average only 0.5 and 2.7% of TDL labeled, respectively, with blocked anti-light chain and blocked anti-polyheavy chain IgG.

The thymus and TTDL suspensions labeled in a different manner (Table I). There was essentially no labeling on short exposures with any radioiodinated anti-immunoglobulin IgG. On prolonged exposure, however, labeling was observed with anti-light chain IgG but not with the anti-heavy chain IgG. The percentage of TTDL labeled with anti-light chain IgG was greater than the percentage of thymus cells labeled (37.0% average *versus* 14.4% average).

Each experiment in Table I was performed as a unit with the simultaneous incubation of thymus, TDL, and TTDL suspensions with the identical freshly radioiodinated IgG. The binding of the anti-heavy chain IgG (and the lack of binding with the blocked IgG) could therefore be observed on TDL even though under identical conditions no labeling was observed on TTDL and thymus cells.

Grain Counts on Labeled Cells.—The grain counts of cells with either unblocked or blocked radioiodinated IgG were compared. The thymus cell suspensions in experiment 2 were tabulated according to the number of grains per labeled cell (Fig. 1). With the unblocked anti-light chain IgG, a total of 302 cells had to be observed to find 100 labeled cells. Accordingly, 302 were observed with the blocked IgG-treated thymus cell suspension. The difference in grain distribution was marked. There were many cells (19%) with greater than 15 grains/cell in the unblocked IgG suspension but very few with a similar grain count (2.0%) with the blocked IgG. Similarly the TTDL suspension from experiment 2 was examined (Fig. 1). In this case only 145 cells had to be observed to tabulate 100 labeled cells. The grain distribution of thymus and TTDL were similar. Again it was apparent that the difference between cell suspensions labeled with unblocked and blocked materials was in the cells bearing 15 or more grains/cell (44.7% versus 1%, respectively). The anti-heavy chain IgG produced no significant labeling of TTDL or thymus cells (less than 10% had 1–6 grains/cell) and there was no difference between blocked and unblocked anti-heavy chain preparations.

Size Distribution of Labeled Thymus and TTDL.—It was observed that the labeled cells in the thymus suspension were predominantly large cells. Therefore, the thymus cells were divided into two groups; one group comprised those cells greater than 10 μ in diameter and the other group those less than 10 μ (Fig. 2). Whereas only 25% of all thymus cells were greater than 10 μ , 79% of the labeled cells were in this category.

The TTDL cells were evaluated in the same manner (Fig. 2). The total suspension was mainly comprised of larger cells (76% greater than 10 μ) and the label was distributed equally between the larger and smaller cells.

DISCUSSION

The results of the above experiments directly demonstrate the presence of immunoglobulin light chains on the surface of thymus and thymus-derived lymphoid cells. Previous studies on the question of the presence of surface immunoglobulins have been performed mainly on mixed B cell and T cell populations or have reported negative results for thymus cells. These studies have used a variety of techniques to investigate surface immunoglobulins. Some workers have used blast transformation by anti-allotype sera (20) or anti-immunoglobulin sera (21). Other approaches have included the inhibition of rosette-forming cells (RFC) by anti-immunoglobulin sera (22, 23), the inhibi-

tion of the binding of radioiodinated antigen on the antigen-sensitive cell by anti-immunoglobulin sera (5), and the formation of rosettes with mouse lymphocytes and heterologous erythrocytes coated with mouse immunoglobulin in a

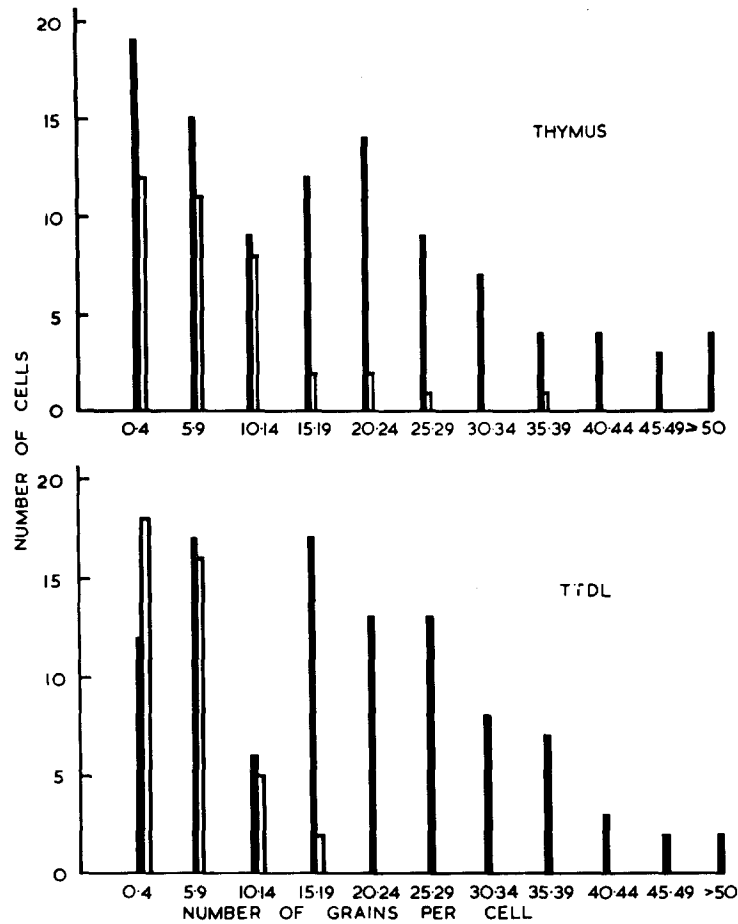


FIG. 1. Distribution of grain counts on labeled cells from thymus and TTDL. Solid bar represents grain counts with anti-light chain protein and open bar represents grain counts with the same protein absorbed (blocked) with purified light chain. 302 and 145 cells from experiment 2 after long exposures were counted, respectively, for thymus and TTDL.

Coombs'-type reaction (24). Functional approaches have included the inhibition of responses to a variety of antigens in irradiated mice reconstituted with spleen cells treated with anti- μ sera (5),² and the inhibition of a similar response

² Herrod, H., and N. L. Warner. Inhibition by anti- μ chain sera of the cellular transfer of antibody and immunoglobulin synthesis in mice. Manuscript in preparation.

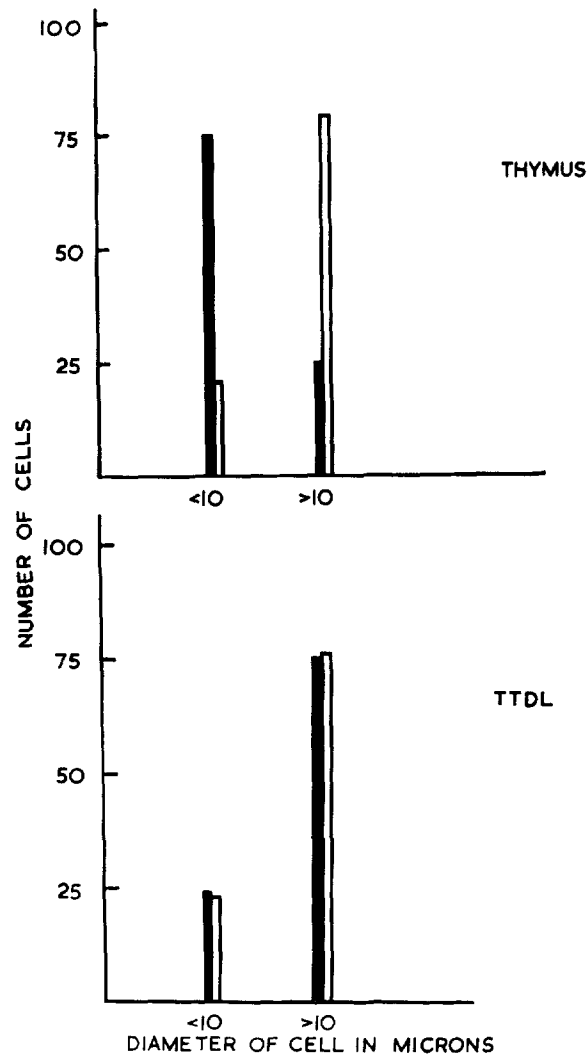


FIG. 2. Distribution of cell diameters on cells from thymus and TTDL. Open bar represents cells labeled with anti-light chain protein (greater than 15 grains/cell) while solid bar represents all cells regardless of label. 100 cells from experiments 1 and 2 after long exposures were averaged to obtain these distributions.

in vitro with anti-light chain sera (25). Experiments on the direct verification of the presence of surface immunoglobulin have used anti-immunoglobulin proteins labeled with radioactive iodine (10, 13) or fluorescein (11, 12).

The above studies have all verified the presence of surface immuno-

globulins on B cells and its presumptive role as an antigen receptor. Heretofore direct evidence of the presence of immunoglobulin on thymus or thymus-derived cells by the binding of anti-immunoglobulin proteins has been lacking. Several investigators have suggested immunoglobulins are present on T cells by the use of indirect techniques such as the inhibition of RFC by anti- μ and anti-light chain sera in a population which was enriched to 85% with theta-positive cells (22). T cell-mediated immune reactions have also been blocked with anti-immunoglobulin sera such as in the inhibition of graft-*versus*-host reactions by anti-light chain sera in neonatal mice (6) and chickens,³ and the inhibition of graft-*versus*-host reactions in adult, irradiated mice receiving parenteral spleen or bone marrow cells (7). Only anti-light chain sera were observed to block the reaction in the neonatal mice or chickens, whereas only anti-globulin sera with broad specificity was used in the other experiments (7). Anti-light chain serum has also been reported to block the antigen-¹²⁵I-induced suicide of T cells (4).

The experiments described in this present paper report on two lymphoid cell populations, thymus and TTDL, which are exclusively composed of T cells as shown by the presence of the theta marker on greater than 95% of the cells (17) and the absence of a B cell receptor for antigen-antibody complexes (26). With these cell suspensions a maximum of 19 and 50%, respectively, of thymus and TTDL were labeled with radioiodinated anti-light chain IgG. Such results could not be explained by the presence of B cell contamination. It might be speculated that the true number of T cells with surface light chains might approach 100% with even more prolonged exposures, or alternatively, it may be that only antigen-activated cells or cells which have recently achieved immunocompetence (antigen-sensitive cells) are expressing surface immunoglobulin. This possibility of a young population of antigen-activated cells bearing surface immunoglobulin is reinforced by the finding that 79% of the lymphoid cells labeling in the thymus were large cells greater than 10 μ in diameter. It is well known that such a population of larger cells is correlated with recent mitotic activity (27). Furthermore the TTDL population was comprised mostly of large, blast-like cells which predictably had a larger number of labeled cells than was present in the thymus (average of 37% *versus* 14.4% for TTDL and thymus, respectively). Since the medulla of the thymus has a higher percentage of large lymphocytes than the cortex (28), it is interesting to speculate that the labeled thymus cells might correspond to the medullary steroid-resistant population which are particularly active against allogenic cells (29) and are the cells which eventually appear as TTDL.

³ Rouse, B. T., and N. L. Warner. Suppression of graft-*versus*-host reactions in chickens by pretreatment of donor cells with anti-light chain sera. Manuscript in preparation.

On the basis of RFC-blocking studies with specific anti-immunoglobulin sera on primed and unprimed mice, some authors (22) have proposed that T cell antigen activation may involve the progressive exposure of an IgM receptor molecule. The unprimed cell would have only the Fab portion of the molecule exposed while the primed cell would sequentially expose the "hinge region" and, finally, essentially the entire IgM molecule. It should be stressed that the studies reported here do not support this concept, since no labeling by radioiodinated anti-heavy chain reagents was observed on an antigen-activated population (TTDL). There may, however, be a difference in the nature of surface immunoglobulins on the "collaborative" cells involved in the SRBC response and the "killer cell" population of the TTDL activated against the histocompatibility antigen.

No claim is made here concerning the functional role of surface immunoglobulins demonstrated on T cells; however, if one were to speculate that their role is as an antigen receptor, it would perhaps be unlikely that there was not an associated variable part of a heavy chain. Such a statement is based on the poor antigen-binding efficiency of an artificial molecule reconstituted from light chains alone (30) although such an artificial molecule may be a distant approximation of such a configuration in nature. There are several explanations for the absence of at least part of a heavy chain demonstrable with the present techniques. It is possible that the T cell has a heavy chain buried in the surface matrix and unavailable for detection by anti-immunoglobulins; there may be a new, unidentified heavy chain class (IgX); or the antisera prepared against purified myeloma proteins did not possess activities against an exposed hinge region, variable part of a heavy chain, or the monomer of an IgM molecule.

The fact that 19% of TDL label on short exposures correlates well with the data on the incidence of theta-positive lymphocytes (approximately 80%) (31) and suggests that this population labeling rapidly with anti-immunoglobulin IgG is a B cell population. The results of cell labeling with anti-immunoglobulin reagents on TDL from neonatally thymectomized mice (10) and TDL from mice with the congenital absence of a thymus⁴ also suggest that the rapid labeling cells are B cells. In contrast to this, the T cell population did not label unless exposed for 1 month or longer and suggests a difference in immunoglobulin surface density between the B and T cell. Although no attempt was made to correlate grain count with surface molecular density, other studies (10, 11) have suggested a difference between the T cell and B cell in the range of 0.1–1.0%. The single experiment performed on TDL with anti-light chain IgG, anti-polyheavy chain IgG, and anti- μ IgG (17.9, 20.2, and 17.6%, respectively) suggests that there are multiple heavy chains present on lymphoid cells at some stage of their development. This has been suggested in several studies

⁴ Bankhurst, A. D., and N. L. Warner. Unpublished observations.

(10, 20, 23). It is not clear why there is not a subpopulation in TDL representing the T cells which will become labeled after prolonged exposure like thymus or TTDL. It is conceivable that the small lymphocytes comprising such a T cell population have a much denser surface matrix of unrelated materials that would submerge the surface immunoglobulins and make them inaccessible for detection. Some support for this concept comes from work done on the greater density of histocompatibility antigens on mouse lymphocytes from lymph nodes or spleen *versus* thymus (32).

The possibility that cytophilic immunoglobulin accounts for the observed results cannot be rigorously excluded despite extensive efforts at cell washing. This is not likely since heavy chain labeling would also be expected if this were the case. In addition there could conceivably be other surface antigens which cross-react with the anti-immunoglobulin IgG.

SUMMARY

Lymphoid cells from thymus, thoracic duct lymph (TDL), and thoracic duct lymph in irradiated animals reconstituted with allogeneic thymus cells (TTDL) were labeled with radioiodinated anti-immunoglobulins using radioautographic techniques. Thymus and TTDL were labeled (14.4 and 37.0%, respectively) with anti-light chain protein after prolonged exposures (30–60 days). No labeling was observed on thymus and TTDL with anti-polyheavy chain globulin. In contrast 18.5–19.0% of TDL labeled on short exposure (6 days) with anti-polyheavy chain and anti-light chain materials. It is proposed that the difference between the labeling observed on short exposures *versus* long exposures can be related to the difference in surface density of immunoglobulins between nonthymus-derived (B) and thymus-derived (T) cells.

The distribution of labeled cells in the thymus was preferentially among the larger cells (greater than 10 μ diameter). The TTDL population was mostly composed of a larger, blast-like population and the distribution of label was independent of size.

As the thymus and TTDL preparations contain almost exclusively T cells, this represents a direct demonstration of surface immunoglobulin light chains on T lymphoid cells.

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