# IMMUNOGLOBULIN MOLECULES ON THE SURFACE OF ACTIVATED T LYMPHOCYTES IN THE RAT*

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A central problem in cellular immunology is the elucidation of the nature of the antigen receptor molecules on the surface of thymus-dependent lymphocytes (T cells). Antigen receptors on thymus-independent lymphocytes (B cells) are readily detectable and have been identified as being mainly monomeric IgM molecules (1–6). However direct attempts to demonstrate immunoglobulin (Ig) molecules on the surface of T cells by immunofluorescence, cytotoxic, or autoradiographic methods have either been unsuccessful or have detected only small amounts of immunoglobulin (5–9). This is consistent with results of experiments that indicate that T cells have many fewer antigen-binding sites than do B cells (10). Indirect evidence in support of the notion that Ig molecules act as antigen-recognition sites on T cells has come from the demonstration that in some instances anti-Ig sera can interfere with antigen-binding and certain functions ascribed to T cells (11–15).

In both the direct and indirect experiments only immunoglobulin light chain determinants have been detected with any consistency on T cells. This has led some investigators to postulate that the antigen receptors on T cells are immunoglobulin molecules that possess unique heavy chain determinants ("IgX") (16).[1] Still others have suggested the possibility that the antigen receptors on T cells are not immunoglobulin molecules at all, but an as yet undescribed product of the immune response (Ir) genes of the major histocompatibility locus (17).

Recently, Marchalonis et al. (18), using an enzymatic radioiodination method to label surface proteins on living lymphocytes (19), have reported that monomeric IgM molecules serve as antigen receptors on human and mouse T cells. Surprisingly, they found that T and B cells had approximately the same number of IgM molecules on their surfaces. They also presented evidence to support the hypothesis, earlier proposed by Greaves and Hogg (20), that Ig molecules on T cells, but not on B cells, tend to be wholly or partially masked. According to this view, only a small number of

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[1] *Abbreviations used in this paper:* BSS, balanced salt solution; Con-A, concanavalin A; IgX, immunoglobulin molecules that possess unique heavy chain determinants; Ir, immune response; PBS, phosphate-buffered saline; PHA, phytohemagglutinin; PWM, pokeweed mitogen; SRBC, sheep red blood cell; TCA, trichloroacetic acid; TxBM, thymectomized, irradiated, and bone marrow-reconstituted rats.

Ig molecules on T cells are normally available to be detected by antigens, anti-Ig sera, and perhaps by certain chemical extraction procedures (21).

A variety of studies have indicated that antigen-stimulated T cells are more efficient than nonstimulated T cells at implementing contact-mediated target cell destruction (effector cells) (22, 23), cooperating with B cells in humoral antibody production (helper cells) (24–27), and initiating accelerated cell-mediated immunological responses (memory cells) (28). All of these acquired functions require antigen recognition. The present experiments were designed therefore to determine whether immunoglobulin molecules are more readily detectable on activated than on nonactivated T cells; and if so, whether the stimulus for T cell activation need be immunologically specific.

## *Materials and Methods*

*Animals.*—2- to 3-mo old male and female Lewis (Le), DA, BN, and ACI strain rats, and the (Le × DA)$F_1$ and (Le × BN)$F_1$ hybrid crosses were used as donors of lymphocytes. 3- to 5-day old male and female Le, ACI, and DA strain rats and the (Le × DA)$F_1$ hybrid cross were used as donors of kidney cells.

*Preparation of Lymphocytes.*—Lymphocytes were collected from the thoracic duct of male rats for 16–20 h at room temperature into sterile flasks containing 5 ml Krebs-Ringer solution, 100 U heparin, and 500 μg streptomycin (29). Lymphocytes were also obtained from the popliteal lymph nodes of tuberculin-sensitized rats by expression of the cells into Tyrode's solution or Hanks' balanced salt solution (BSS). The lymphocytes were washed twice in Tyrode's solution by centrifugation (10 min × 100 *g*; 4°C) and suspended in culture medium at a concentration of 2 or 15 × $10^6$ cells/ml.

*Preparation of Kidney Monolayer Cultures.*—Kidneys were cut into small pieces in Hanks' BSS and trypsinized (0.25%) for 45 min at 37°C with gentle agitation. The resulting cell suspension was washed once and suspended in Waymouth's medium containing 10% heat-inactivated newborn calf serum, 100 U penicillin/ml, and 100 μg streptomycin/ml. Approximately 2.5 × $10^5$ kidney cells in 2 ml medium were placed in 10 × 35 mm plastic petri dishes (Falcon Plastics, Div. of BioQuest, Oxnard, Calif.) and incubated at 37°C in 5% $CO_2$ in air for 5–7 days until the monolayers reached confluency.

*Sensitization of Rats.*—Adult Le rats were injected in the hind footpad with 0.3 ml complete Freund's adjuvant (Difco Laboratories, Detroit, Mich., H37 Ra) containing 300 μg *Mycobacterium tuberculosis* (30). Lymphocytes were collected from the draining popliteal lymph node 9 days after immunization.

*Antisera.*—Rabbit IgG fraction against rat gamma globulin and fluorescein-conjugated goat IgG fraction against rabbit IgG were checked for specificity by immunoelectrophoresis against whole rat or rabbit serum using purified rat gamma globulins or rabbit IgG as standards (Cappel Laboratories, Downingtown, Pa.). Except where noted, the rabbit antirat Ig serum was used in a dilution of 1:20, at which it selectively stained (by indirect immunofluorescence) B lymphocytes from thymectomized, irradiated (1,000 R), and bone marrow-reconstituted (TxBM) rats (31), but did not stain thymocytes or T lymphocytes in the thoracic duct lymph of normal rats. The fluorescin-conjugated goat antirabbit IgG serum was used in a dilution (1:20) at which it did not react detectably with rat lymphocytes.

Antiserum to rat thymus-dependent lymphocytes (anti-T cell serum) was prepared by injecting rabbits intravenously on two occasions 10 days apart with suspensions of $10^8$ viable thoracic duct lymphocytes from Le strain rats (32). The antiserum obtained 7 days after the second injection was extensively absorbed with rat erythrocytes, casein-induced peritoneal

exudate cells, and splenic lymphocytes from TxBM rats. After absorption, the anti-T cell serum reacted with thymocytes and with lymphocytes in "thymus-dependent" areas (29) of lymph node and spleen, but not with B cells. The anti-T cell serum had no detectable activity against rat gamma globulins as determined by immunoelectrophoresis, nor was its reactivity against T cells inhibited by absorption with rat gamma globulin.[2]

Antisera to histocompatibility antigens were prepared by injecting Le strain (AgB-1) rats intravenously at 2-wk intervals with 2–4 doses of $10^8$ viable DA strain (AgB-4) or BN strain (AgB-3) rat spleen cells. The rats were bled 7 days after the last injection, and the antisera were cross-absorbed with BN or DA strain spleen cells. Antiserum to Le strain histocompatibility antigens was prepared in BN rats and absorbed with DA spleen cells.

*Activation of Lymphocytes.*—With the exception of the tuberculin stimulation study, thoracic duct lymphocytes were used in all experiments. The culture medium consisted of Eagle's basal medium, 1% L-glutamine, 10 or 20% heat-inactivated (56°C × 30 min) rat, human, or horse serum, 100 U penicillin/ml, and 100 μg streptomycin/ml. The cultures were incubated in 16 × 125 mm round bottom culture tubes (except for the in vitro allograft reaction) at 37°C in an atmosphere of 5% $CO_2$ in air.

*Mixed lymphocyte reaction:* $2 \times 10^6$ Le and $2 \times 10^6$ (Le × DA)$F_1$ or (Le × BN)$F_1$ strain lymphocytes were mixed in 2 ml medium (10% rat or human serum) and incubated 5 days. Syngeneic controls were included in each experiment.

*In vitro allograft reaction:* $30 \times 10^6$ Le strain lymphocytes in 2 ml Dulbecco's modified Eagle's medium containing 20% horse serum were added to confluent monolayers of neonatal syngeneic, allogeneic, or hemiallogeneic rat kidney cells. The cultures were incubated 5 days, after which the lymphocytes were harvested from the monolayers by gentle washing with Hanks' BSS. The presence of target cell destruction was subjectively evaluated on toluidine blue-stained monolayers. The optimal conditions for target cell destruction have been quantified in previous experiments (33).[3]

*Stimulation with tuberculin:* $2 \times 10^6$ Le strain lymphocytes from the popliteal lymph nodes of tuberculin-sensitized rats were suspended in 1 ml medium (20% rat or human serum) in the presence or absence of 20 μg purified tuberculin (Microbiological Research Corp., Bountiful, Utah) and incubated 4 days.

*Stimulation with mitogens:* $2 \times 10^6$ Le strain lymphocytes were suspended in 1 ml medium (20% rat or human serum) in the presence of 5 μg phytohemagglutinin (purified PHA; Wellcome Reagents Ltd), 0.05 ml pokeweed mitogen (PWM; Grand Island Biological Co., Grand Island, N. Y.), or 10 μg concanavalin A (Con-A, Difco), and incubated 4 days. Unstimulated control cultures were included in each experiment. Optimal concentrations of mitogens were determined in preliminary experiments.

*Enumeration of Activated Lymphocytes.* –

*Morphological criteria:* Percentages of large (activated) and small lymphocytes were determined on methyl green-pyronin stained smears of lymphocyte suspensions. Lymphocytes less than 8 μm diameter were designated "small" lymphocytes, and those greater than 8 μm diameter as "large" lymphocytes. Most of the large lymphocytes had intensely pyroninophilic cytoplasm. Dead cells, which appeared as pale staining ghosts without nuclear detail, were not counted.

In the immunofluorescence assay (*vide infra*) the numbers of specifically fluorescing large and small lymphocytes were expressed as percentages of total lymphocytes in each size class. Total large and small lymphocytes were determined under dark-field illumination using a calibrated eyepiece micrometer. After correction for dead cells (identified by their diffuse

[2] A detailed description of the method of preparation of the anti-T cell serum and the assays for specificity is in preparation.

[3] Cogen, R. B., and R. R. Lindquist. Sensitization of lymphocytes to the presence of non-lymphoid cells in culture. Manuscript submitted for publication.

cytoplasmic fluorescence), the totals were found to be comparable proportionately to those derived from counts of cells in stained smears.

*DNA synthesis:* 1 μCi tritiated thymidine ([*methyl*-$^3$H]thymidine, 6.7 Ci/mmol, New England Nuclear, Boston, Mass.) was added in 0.1 ml Hanks' BSS to cultures of lymphocytes 18 h before harvesting. At the end of the incubation period, the cells were collected by centrifugation (150 *g* × 10 min), washed twice in cold Tyrode's solution, and extracted in three changes of 5% cold trichloroacetic acid (TCA) (total 6 ml). The precipitate was drained for 30 min at 4°C, solubilized in 0.2 ml formic acid, and the radioactivity was measured in a liquid scintillation counter (Nuclear Chicago, Des Plaines, Ill.). Results were corrected for background radioactivity and quenching.

*Immunofluorescence.*—Indirect immunofluorescence was performed on suspensions of viable lymphocytes at 4°C using techniques described previously (34). Briefly, the cells were washed twice in cold Tyrode's solution and suspended in Dulbecco's phosphate-buffered saline (PBS, pH 7.4). Approximately $10^6$ cells in 0.05 ml PBS were mixed with 0.05 ml rabbit antiserum (final dilution 1:20) in a U-well microtiter tray and incubated for 20 min at 4°C. The cells were washed in the tray three times with cold PBS and incubated (20 min; 4°C) in 0.025 ml of a 1:20 dilution of fluorescein-conjugated goat antirabbit IgG. After rewashing three times in cold PBS, samples were withdrawn from each well and examined immediately under dark-field–fluorescence illumination. Normal rabbit serum controls were included in each experiment. Other controls are described under Results.

## RESULTS

Lewis strain rat thoracic duct lymphocytes were stimulated to undergo blast transformation in vitro by exposing them to specific antigens or to mitogens. The surfaces of the transformed lymphocytes and of nontransformed control lymphocytes were examined by indirect immunofluorescence for the presence of specific T cell antigens ($T^+$) and for readily detectable immunoglobulin molecules ($Ig^+$). A summary of results, representing the mean values of 2 to 5 experiments, is presented in Table I.

*Nonstimulated Lymphocytes.*—On day 1 of culture an average of 12% of small lymphocytes were $Ig^+$ and 85% were $T^+$; by days 4–5 of culture less than 1% of lymphocytes were $Ig^+$ and more than 95% were $T^+$. The $Ig^+$ cells were presumed to be B cells. Inasmuch as the absolute numbers of both $Ig^+$ and $T^+$ lymphocytes decreased during the incubation period, the change in proportionate representation of the two cell types presumably reflected a more rapid decrease of B cells relative to T cells, an observation which has been reported previously (35). Some of the large lymphocytes in the unstimulated lymphocyte cultures were $Ig^+$ and some were $T^+$, but there were too few large cells to determine whether these represented separate or overlapping populations.

*Antigen-Stimulated Lymphocytes.*—Lymphocytes which were induced to undergo blast transformation by exposure in vitro to purified tuberculin or to foreign histocompatibility antigens (mixed lymphocyte and in vitro allograft reactions) were almost exclusively T cells (Fig. 1 *a*). The small lymphocytes, which comprised the majority of cells in these cultures, were also mainly T cells (Fig. 1 *b*). However, 70–92% of the large lymphocytes in antigen-stimu-

TABLE I

*Surface Antigens of Activated Lewis Rat Lymphocytes as Determined by Indirect Immunofluorescence*

| Conditions of culture | | | Lymphocyte transformation* | | Large and small lymphocytes reacting with antisera to rat* | | | |
|---|---|---|---|---|---|---|---|---|
| Method of lymphocyte activation | Incubation | Serum supplement‡ | Large lymphocytes | Increase in [$^3$H]-thymidine uptake§ | T lymphocytes | | Immunoglobulin | |
| | | | | | Large | Small | Large | Small |
| | *days* | | % | | % | | | |
| —‖ | 4 or 5 | 20% human or horse | <5 | — | — | >95 | — | <1 |
| Mixed lymphocyte reaction¶ | 5 | 10% human | 20 | 16 | 87 | >95 | 70 | 2 |
| In vitro allograft reaction** | 5 | 20% horse | 24 | — | >95 | >95 | 87 | <5 |
| Purified tuberculin‡‡ | 4 | 20% human | 13 | 7 | >95 | >95 | 92 | 1 |
| Phytohemagglutinin | 4 | 20% human | 75 | 10 | >95 | >95 | <1 | <1 |
| Concanavalin A | 4 | 20% human | 67 | 32 | >95 | 92 | 2 | 3 |
| Pokeweed mitogen | 4 | 20% human | 71 | 16 | 72 | 77 | 24 | 28 |

* Each entry is the mean of 2–5 experiments. Large lymphocytes have cell diameter greater than 8 μm.

‡ All experiments were repeated using normal rat serum as the serum supplement. There was no significant difference in results when normal rat serum was substituted for human or horse serum, except that the degree of lymphocyte transformation was usually less.

§ Each entry is the ratio of [$^3$H]thymidine incorporation (18-h period) in cultures of stimulated and control lymphocytes.

‖ Syngeneic and nonstimulated control cultures.

¶ Lewis strain lymphocytes were reacted against (Le × DA)$F_1$ or (Le × BN)$F_1$ strain lymphocytes. After 5 days incubation, 95% of the large lymphocytes and 86% of the small lymphocytes were of Lewis origin as determined by immunofluorescence using antisera to Le, DA, and BN histocompatibility antigens.

** Lewis strain lymphocytes were reacted against DA, ACI, or (Le × DA)$F_1$ strain rat kidney cells.

‡‡ Lymphocytes, obtained from popliteal lymph node of rats injected in footpad with Freund's complete adjuvant 9 days previously, were incubated in vitro with purified tuberculin.

lated cultures were $Ig^+$, whereas more than 95% of the small lymphocytes were $Ig^-$ (Fig. 2 *a*, *b*).

It was noted that large lymphocytes more than 15 μm in diameter tended to be $Ig^-$, whereas large lymphocytes 10–14 μm in diameter were usually $Ig^+$. Large lymphocytes in both size classes were $T^+$.

The fluorescent staining on the $Ig^+$ blast transformed T cells was usually distributed in a patchy, circumferential pattern (Fig. 2 *a*, *b*). "Capping" (36) of the surface Ig was not seen under the temperature conditions (4°C)

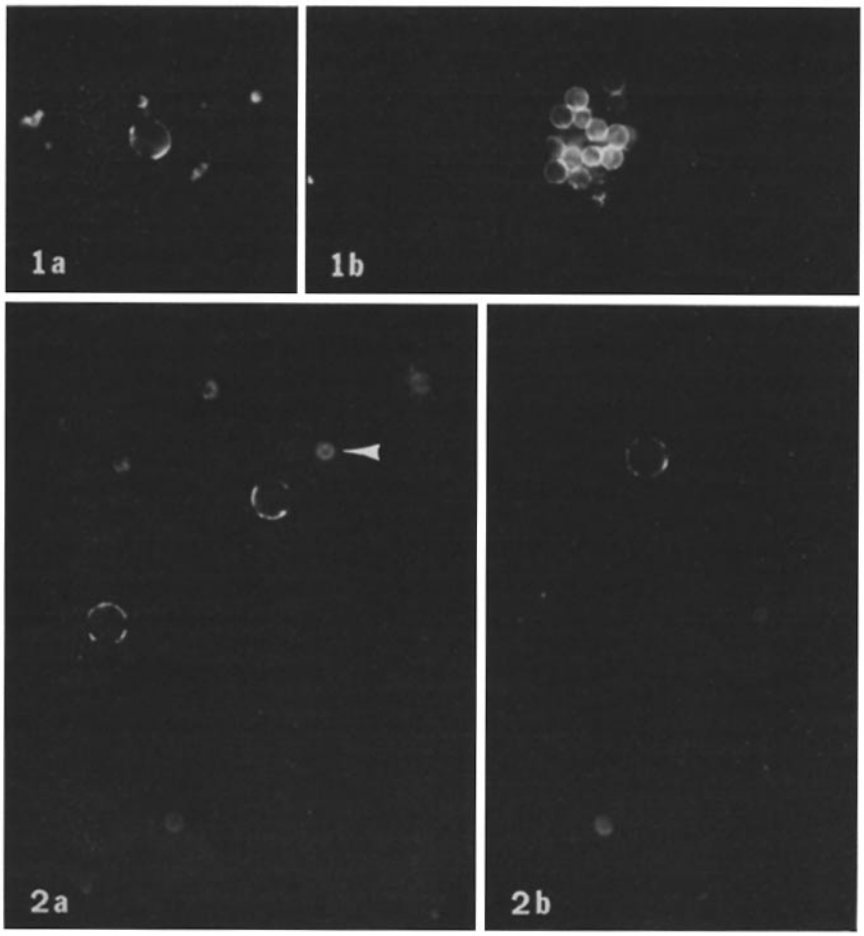


FIG. 1. Suspension of rat lymphocytes from mixed lymphocyte reaction [Le + (Le × DA)$F_1$; 5 days' incubation] exposed to rabbit antirat T cell serum, washed, and reacted with fluorescein-conjugated goat antirabbit IgG. × 400. (*a*) Large lymphocyte with specific surface immunofluorescence. Bright spots in same field represent cellular debris. (*b*) Agglutinated small lymphocytes with specific surface immunofluorescence.

FIG. 2. Suspension of rat lymphocytes from mixed lymphocyte reaction [Le + (Le × DA)$F_1$; 5 days' incubation] exposed to rabbit antirat Ig serum, washed, and reacted with fluorescein-conjugated goat antirabbit IgG. × 400. (*a*) Two large lymphocytes with bright green immunofluorescence sharply delineating their surfaces. Viable small lymphocytes in the same field appear as pale grey ghosts. One small lymphocyte (arrow) has diffuse green cytoplasmic fluorescence, suggesting that it is a dead or injured cell. (*b*) Specifically fluorescing large lymphocyte and two nonfluorescing small lymphocytes.

at which the fluorescence studies were done. The intensity of fluorescence of large T cells treated with anti-Ig serum was less than that of small B cells similarly treated.

*Mitogen-Stimulated Lymphocytes.*—The degree of blast transformation was considerably greater in mitogen-stimulated cultures (67–75%) than in antigen-stimulated cultures (13–24%). More than 95% of the large lymphocytes in the PHA- or Con-A-stimulated cultures carried T cell antigens. In contrast to results obtained with antigen-stimulation, less than 5% of T cells stimulated with PHA or Con-A had detectable immunoglobulins on their surface.

In order to exclude the possibility that mitogen-stimulated T cells expressed surface immunoglobulins at times other than those seen for antigen-stimulated T cells (4–5 days), lymphocytes from Con-A-treated cultures were examined on days 0–8. Although blast transformation was apparent on the second day of culture and was maximum by the third day, increased numbers of $Ig^+$ large lymphocytes were not seen in any of the samples. Immunoglobulin-bearing small lymphocytes (B cells) decreased in number with time, as in nonstimulated control cultures.

Results of PWM-stimulation differed from those obtained with PHA and Con-A. Only 75% of both large and small lymphocytes in the PWM-stimulated cultures were $T^+$, and approximately 25% were $Ig^+$. PWM has been reported to stimulate both B and T lymphocytes in the mouse (37). Results of the present experiments suggest that it also may do so in the rat. Thus, when PWM-stimulated T cells were exposed to a mixture of equal parts anti-T cell and anti-Ig sera approximately 90% of the large lymphocytes were positive, suggesting that at least 18% of the activated lymphocytes were B cells ($T^-Ig^+$). Of equal importance, it could be deduced that at least 92% of the activated T cells were $Ig^-$.

*Controls.*—

*Specificity of anti-immunoglobulin serum:* Despite the specificity for rat gamma globulins demonstrated by immunoelectrophoresis, it was possible that the anti-Ig serum contained "natural" antibodies to nonimmunoglobulin antigens on the surface of activated T cells. This was excluded by the fact that all reactivity of the anti-Ig serum for both small B cells and antigen-stimulated T cells could be removed by absorption with rat gamma globulin. In addition, two other lots of rabbit anti-rat Ig sera also reacted positively with antigen-stimulated T cells.

The specificity of the anti-Ig serum for antigen-stimulated T cells was confirmed by demonstrating a marked difference between the fluorescent antibody titers against antigen-stimulated T cells (titer = 1:128) and against mitogen-stimulated or nonstimulated T cells (titers < 1:2).

*Cytophilic antibodies:* The possibility that cytophilic antibodies from serum in the media in which the lymphocytes were cultured accounted for the Ig molecules on antigen-stimulated T cells was excluded by the following

observations. Antigen-stimulated T cells were $Ig^+$ whether they had been cultured in the presence of homologous (rat) or heterologous (human, horse) sera. The antisera used to detect surface Ig did not cross-react with human or horse gamma globulins as shown by immunoelectrophoresis and by absorption studies. This is not to say that antibodies in the heterologous serum supplement did not attach to rat lymphocyte surfaces, but only that they could not be detected with the rabbit antirat Ig serum or the fluorescein-conjugated goat antirabbit IgG serum. Indeed, when rabbit antihuman Ig serum was used in lymphocytes cultured in human serum, human Ig was identified on the surface of nonstimulated, antigen-stimulated and mitogen-stimulated T cells.

*Masking of surface Ig by mitogens:* It could be argued that the inability to detect immunoglobulin molecules on mitogen-transformed T lymphocytes was due to a masking effect of the mitogen. This does not appear to be the case, as the following experiments show.

An appropriate concentration of PHA was added on the fifth day of culture to cells undergoing a mixed lymphocyte reaction. Agglutination of the cells attested to the presence of PHA on their surface. 1 h after addition of the PHA the lymphocytes were harvested, washed as usual, and examined by indirect immunofluorescence. Immunoglobulin molecules were detected as readily on the PHA-treated cells as on untreated cells from mixed lymphocyte cultures.

Similarly, exposure of freshly-collected thoracic duct lymphocytes to PHA or Con-A for 1 h did not prevent the anti-Ig serum from reacting with B lymphocytes. Also, anti-T cell serum and antihistocompatibility antigen sera reacted as strongly with mitogen-stimulated lymphocytes as with antigen-stimulated lymphocytes.

These observations also argue against the possibility that PHA or Con-A caused redistribution and pinocytosis of Ig molecules on mitogen-stimulated T cells (36).

## DISCUSSION

Results of the present experiments indicate that immunoglobulin molecules are present in increased quantity or in a more readily detectable form on the surface of antigen-stimulated T lymphocytes than on nonstimulated or mitogen-stimulated T lymphocytes. The source of these Ig molecules is not yet known, but it seems clear that they are not derived from the homologous or heterologous sera that were used to supplement the culture media. The simplest explanation is that the Ig molecules are the products of the T cells which carried them. However, the possibility has not been entirely excluded that B cells in the cultures produced cytophilic antibodies, which affixed themselves to antigen-stimulated T cells. This is deemed unlikely in the absence of a demonstrable B cell proliferative response in the antigen-stimulated cultures. Furthermore, in cultures of PWM-stimulated lymphocytes, which

potentially contained a large number of antibody-secreting B cells (37), at least 92% of the activated T cells were $Ig^-$. Of course it is possible that cytophilic antibodies from B cells were unable to bind to mitogen-coated T cells. Experiments using pure T cell suspensions or mixed populations of B and T cells from donors with different allotypic markers on their immunoglobulins should help to resolve this problem.

Another possible explanation for the observed results is that rabbit or goat Ig molecules from the antisera used in the indirect immunofluorescence assay mimicked rat Ig molecules on the surface of antigen-stimulated T cells. Such Ig molecules in the form of cytophilic antibodies, aggregated gamma globulin, or antigen-antibody complexes might have attached nonspecifically to the activated T cells, perhaps to Fc receptors. While it was clear from control experiments that the fluorescein-conjugated goat antirabbit IgG did not react directly with activated rat T cells, we could not completely exclude the possibility that the rabbit antirat Ig serum interacted nonspecifically with these cells. However, this seems extremely unlikely in view of the finding that all reactivity of the rabbit antiserum for activated T cells could be removed by absorption with rat gamma globulin. Furthermore, the process of absorption itself created immune complexes which would have been expected to adhere to T cells under the postulated circumstances; yet these complexes were not found on the surfaces of the activated T cells.

Several other studies have indicated that blast transformed T cells have more readily detectable surface Ig molecules than nontransformed T cells. Biberfeld et al. (38) and Hellstrom et al. (39), using immunofluorescence and ferritin-labeling techniques, reported that PHA-, Con-A-, and PPD-stimulated human blood lymphocytes and lymphocytes undergoing a mixed lymphocyte reaction were stained by higher dilutions of antilight chain sera than were nonstimulated lymphocytes. Their results differ from ours in the rat and from those of Jones and Roitt (35) in the rabbit and mouse in that they found that mitogen-stimulated human lymphocytes were stained by anti-Ig reagents. The explanation for this disparity is not clear, although specific markers were not available in the studies of human lymphocytes to definitely identify the $Ig^+$ lymphocytes as T cells.

The point is important. If increased quantity or availability of surface Ig molecules is necessary for thymus-dependent lymphocytes to initiate immunologically-specific reactions, then our results would suggest that only antigen can direct the differentiation of T cells to a state of immunocompetence. This concept is reminiscent of that originally proposed by Ehrlich (40) for immunoglobulin secreting cells. Mitogen-stimulated and otherwise nonspecifically activated T cells, on the other hand, could participate in (as opposed to initiating) immunological reactions by releasing mediators and by effecting nonspecific contactual killing of target cells to which they have been coagglutinated.

It would be of obvious interest to know whether antigen-activation of T

cells in vivo also results in the production or expression of increased surface Ig. The results to date are limited and somewhat conflicting. Roelants (10) found a progressive increase over a 6 mo period in the amounts of antigen that T cells from mice immunized with *Maia squinado* hemocyanin could bind to their surface. It is not known whether this was due to the appearance or selection of T cells carrying an increased number of surface Ig molecules or Ig molecules of higher average affinity for antigen. In another system, Nossal et al. (4) were unable to detect increased surface Ig on "H-2-activated" or sheep red blood cell- (SRBC)-activated mouse thymocytes using a sensitive autoradiographic technique; and Hunter et al. (41) failed to find increased numbers of rosette-forming cells among SRBC-stimulated mouse thymocytes. On the other hand, Greaves and Moller (42) found rosette-forming thymocytes in mice 3 days after SRBC injection but not at 6 days, suggesting that timing may be of importance in detecting Ig on T cells. The T cell rosettes could be inhibited by antilight chain and anti-$\mu$ chain sera. Lesley et al. (15) were able to selectively inhibit SRBC-"educated" thymocytes from participating in a hapten-specific plaque-forming cell assay by treating them with antilight chain serum and complement.

It was not determined in the present experiments whether immunoglobulin heavy chain determinants are detectable by immunofluorescence on antigen-stimulated T cells, although such studies are in progress. The anti-immunoglobulin serum used here contained antibodies to light and heavy chain determinants (D. Stechschulte, personal communication). However, some information is available that bears on this question. Marchalonis et al. (18), using an enzymatic radioiodination technique to label cell surface proteins (19), isolated monomeric IgM molecules in approximately equal quantities from normal and antigen-stimulated mouse thymocytes. Significantly, the ratio of $^{125}$I-labeling of heavy and light chains isolated from nonactivated thymocytes was 1.22/1 while that from activated thymocytes was 1.95/1. This latter figure is similar to that obtained for immunoglobulins on B cells and is approximately two-thirds the value obtained for free immunoglobulins.

Taken as a whole, the data suggest that surface Ig molecules exist in a masked form on resting T cells, and that they become unmasked after stimulation with antigen. The unmasked Ig molecules can be detected more readily by anti-Ig sera, as in the present experiments, and can bind antigen more efficiently than can masked Ig molecules.

## SUMMARY

Lewis strain rat lymphocytes were exposed in vitro to a variety of specific and nonspecific blastogenic stimuli. The surfaces of the transformed lymphocytes were examined by indirect immunofluorescence for the presence of T cell antigens and immunoglobulin molecules.

More than 90% of lymphocytes that underwent blast transformation after

exposure to foreign histocompatibility antigens (mixed lymphocyte reaction; in vitro allograft reaction), purified tuberculin, phytohemagglutinin (PHA), and concanavalin A (Con-A) had T cell antigenic markers on their surfaces. 70–92% of the antigen-stimulated blast cells also had readily detectable surface immunoglobulin molecules, whereas less than 3% of the PHA- and Con-A-activated cells were $Ig^+$. Pokeweed mitogen (PWM) appeared to activate both B and T cells, but the T cells did not have detectable surface immunoglobulin molecules. Nonactivated control cultures contained $T^+Ig^-$ lymphocytes almost exclusively.

The results suggest that thymus-dependent rat lymphocytes express increased amounts of detectable immunoglobulin on their surface in response to specific stimulation with antigen. It is postulated that the acquisition of immunological competence by activated T cells may be related to this expression of surface immunoglobulin.

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