CELL MEMBRANE PERTURBATION OF RESTING T CELLS AND THYMOCYTES CAUSES DISPLAY OF ACTIVATION ANTIGENS

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Functional specialization of cells is manifest, among other things, by the presence in the cell membrane of molecules with distinct functions. These molecules may, in some instances, be detected by antibodies and, in this case, are termed differentiation antigens. In both man and mouse the progressive differentiation of T cells into functionally discrete sets can be followed with antibodies recognizing distinctive displays of differentiation antigens (1–3). In man, the differentiation of T cells from bone marrrow precursors, through the thymus and on to peripheral T cells, can be enumerated with murine monoclonal antibodies, such as OKT1, 3, 4, 6, 8, 10, and 11 (4–6). It is presumed that the controlled expression or de-expression of the molecules recognized by these antibodies is a manifestation of altering gene programs during differentiation. Prothymocytes are a cell type defined by their commitment to T cell differentiation, and these cells can be induced in vitro and in vivo by thymopoietin and other inducing agents to express cell surface antigens characteristic of thymocytes (7). Although it has not been proven formally that the newly expressed antigens are synthesized de novo, this display of differentiation antigens following induction requires energy and both RNA transcription and translation (8).

Another class of cell surface antigens, termed activation antigens, delineate the state of activation of mature T cells rather than functional subdivisions. These include the antigens recognized by the monoclonal antibody OKIa1, which has been found to represent a nonpolymorphic epitope on a molecule corresponding to a product of the immune-associated region (9); OKT9, which is a transferrin binding glycoprotein (10); and OKT10, the function of which is not yet known (11). These antigens are minimally represented on resting peripheral T cells although the antigens recognized by OKIa1 and OKT10 are expressed on resting cells of other lineages (12); all three antigens are displayed on T cells either following in vitro activation or in vivo during the course of certain diseases associated with T cell activation (10, 13–17).

In the course of examining the coexpression of two surface antigens on resting peripheral T cells, using double staining (indirect and direct) with two monoclonal antibodies, we unexpectedly found high levels of certain antigens typically associated with T cell activation. Careful analysis of our methods convinced us

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that this surprising observation was due to actual expression of these "activation antigens" on resting cells and not to a more trivial technical explanation. Apparently, cell membrane perturbation triggered by cross-linking of the first antibody caused the expression of the activation antigens, suggesting that these molecules already existed in the cells and that the expression of "activation" antigens on "resting" cells did not require their de novo synthesis but rather was related to their orientation in the membrane.

Materials and Methods

Monoclonal Antibodies. The derivations of hybridomas producing the monoclonal antibodies designated OKT1, OKT3, OKT4, OKT6, OKT8, OKT9, OKT10, OKT11, and OKIa1 have been previously described as have their staining patterns on peripheral T cells and thymocytes (4, 9–11, 18–21). Monoclonal antibody W6/32 reactive with a HLA-A,B,C, framework antigen was purchased from Pel-Freeze Biologicals (Rogers, AR). Monoclonal antibody 42/6, reactive with the transferrin binding site of the transferrin receptor (22), was generously provided by Dr. Ian Trowbridge, Salk Institute. Monoclonal antibody anti-Tac which reacts with the interleukin-2 (IL-2) binding site (23) was a gift of Dr. Thomas Waldmann, National Institutes of Health.

Source of Cells. Human peripheral blood mononuclear cells were isolated from healthy volunteer donors by Ficoll-Hypaque density gradient centrifugation (Pharmacia Fine Chemicals, Piscataway, NJ). T cells were obtained by rosetting the mononuclear cells into erythrocyte rosette-positive (E^+)[1] and erythrocyte rosette-negative (E^-) populations with sheep erythrocytes (SRBC). The E^+ population was ~95% $OKT3^+11^+$. Fractionated populations were stored overnight at 4°C in RPMI 1640 containing 20% fetal calf serum (Grand Island Biological Co., Grand Island, NY). Thymocytes were obtained from children 3 mo to 6 yr of age undergoing corrective cardiac surgery. Fragments of thymus were placed in ice cold RPMI 1640 at the time of removal, shipped on ice and processed the same day.

T Cell and Thymocyte Membrane Perturbation. To determine the effect of T cell membrane perturbation on activation antigen expression, we used a panel of three fluoresceinated monoclonal antibodies, OKIa1, OKT9, and OKT10, which define T cell activation antigens. Resting peripheral human T cells or single cell suspensions of human thymocytes were stained with these antibodies (*a*) alone; (*b*) following incubation with OKT3, OKT4, or OKT8 (peripheral T cells) or OKT6 (thymocytes); or (*c*) following cross-linking of these antibodies by the addition of horse α-mouse IgG (Vector Labs, Burlingame, CA).

Quantitative expression of OKIa1, OKT9, and OKT10 was determined on resting peripheral T cells or thymocytes stained with fluorescein isothiocyanate (FITC) conjugated monoclonal antibodies, FITC-OKIa1, FITC-OKT9, or FITC-OKT10. In brief, 1×10^6 T cells were suspended in 100 μl of phosphate-buffered saline (PBS) containing 5 μg of FITC-conjugated monoclonal antibody, 0.01% sodium azide, and 5% fetal calf serum (FCS). The cells were then incubated on ice for 30 min and subsequently washed two times with PBS and resuspended to 1 ml. Fluorescence analysis was carried out on a model 50H Cytofluorograf (Ortho Diagnostic Systems, Inc., Westwood, MA). Data was compiled and analyzed by an interfacing 2150 computer (Ortho Diagnostic Systems, Inc.).

In order to determine whether the binding of the first monoclonal antibody (OKT3, OKT4, OKT6, or OKT8) induced expression of activation antigens, cells were preincubated with either 0.5 μg of OKT3, OKT4, OKT6, or OKT8 monoclonal antibody for 30 min at 4°C. The cells were washed two times with PBS, resuspended in PBS, and stained with FITC-conjugated monoclonal antibody (see above).

To ascertain whether cross-linking of surface-bound monoclonal antibodies would induce expression of activation antigens, the following procedures were carried out. 1 ×

[1] *Abbreviations used in this paper:* E^+, erythrocyte rosette-positive; E^-, erythrocyte rosette-negative; FCS, fetal calf serum; FITC, fluorescein isothiocyanate; PBS, phosphate-buffered saline; PHA, phytohemagglutinin; SRBC, sheep erythrocyte.

10^6 T cells or thymocytes were incubated in 100 μl of PBS containing 5% FCS, 0.01% sodium azide, and 1 μg of one of the aforementioned monoclonal antibodies. The cells were incubated on ice for 30 min and washed two times with PBS. The cell pellets were resuspended in 100 μl of PBS containing horse α-mouse IgG at a final dilution of 1:20 (previously titrated for saturating concentration), and the cells were incubated on ice for an additional 30 min before washing with PBS. The cells were then resuspended in 100 μl of PBS containing 50 μg of nonfluorescent mouse IgG (Cappel Laboratories, Cochranville, PA) added to saturate free combining sites on the horse α-mouse IgG. The cells were incubated on ice for 20 min after which time 5 μg of fluoresceinated OKIa1, OKT9, or OKT10 antibodies were added without a washing step. Following an additional 20-min incubatioin period on ice, the cells were washed and resuspended in 1 ml PBS containing 5% FCS and 0.01% sodium azide. As an additional control the same experiments were carried out using, as the first antibody, a monoclonal antibody to HLA-A,B,C framework, this representing a surface antigen not restricted to T cells.

Saturation of Free Combining Sites on Horse α-Mouse IgG. Preincubation of lymphocytes with OKT3, OKT4, OKT6, or OKT8 followed by horse α-mouse IgG and a fluoresceinated monoclonal antibody necessitated a blocking step to prevent the fluoresceinated antibody from binding to unoccupied sites on the horse α-mouse IgG molecule. The amount of nonfluorescent mouse IgG needed to fill these sites was determined by incubating 1×10^6 peripheral T cells with 1 μg of OKT3, OKT4, OKT6, or OKT8 in 200 μl of PBS for 30 min on ice. The cells were washed twice in PBS containing 5% FCS and 0.01% sodium azide. 100 μl of a 1:20 dilution of horse α-mouse IgG in PBS plus 5% FCS were then added to the cell pellet. The cells were incubated for 30 min on ice and washed twice as described above. The cells were then incubated with increasing concentrations of nonfluorescent mouse IgG (Cappel Laboratories, Cochranville, PA) for 20 min on ice. Following this incubation and without any wash steps, 5 μg of fluoresceinated mouse IgG (F:P ≃ 5) were added and the incubation was continued for an additional 20 min on ice. The cells were then washed three times with PBS containing 5% FCS and 0.01% sodium azide and resuspended to 1 ml. Fluorescence analysis was carried out on a model 50H Cytofluorograf interfaced with a 2150 computer system as described above.

Transferrin Binding. Binding of transferrin to intact cells (24) was measured by incubating 1×10^6 cells per assay in a total volume of 0.5 ml of RPMI 1640 containing 1% bovine serum albumin with ^{125}I-labeled human transferrin [labeled by chloramine-T iodination (25)] at 37°C for 30 min. The incubation was stopped by chilling on ice following addition of 1 ml of ice cold PBS and centrifuging at 400 *g* for 10 min at 4°C. Both the supernatant and the cell pellet were assayed for radioactivity and percentage radioactivity bound to the cells was calculated. Transferrin binding was determined for resting T cells, T cells on which display of the transferrin receptor had been induced with OKT3 plus horse α-mouse IgG and, as positive controls, OKT9$^+$ Raji cells (a Burkitt lymphoma-derived line) and 3-d phytohemagglutinin (PHA)-activated human peripheral T cells. Specificity of ^{125}I-transferrin binding was confirmed by competition with various concentrations of unlabeled transferrin.

Expression of Interleukin-2 (IL-2) Receptor. The IL-2 receptor, as enumerated by anti-Tac monoclonal antibody, was induced on peripheral T cells by incubation with OKT1 and horse α-mouse IgG. As an independent measure of IL-2 receptors the capacity of these cells to adsorb IL-2 was determined in parallel with similar tests on unperturbed peripheral T cells and 3-d PHA-activated T cells (positive control). 1×10^4–1×10^6 cells were incubated with a predetermined limiting amount of partially purified IL-2 for 1 h at 4°C. The cells were then centrifuged at 4°C for 10 min at 400 *g* and the supernatants were assayed for residual IL-2 activity by adding aliquots to day 7 PHA-stimulated peripheral blood lymphocytes, with measurement of [^{3}H]thymidine incorporation after 48 h.

Results and Discussion

We first determined that the percentage of peripheral T cells stained with FITC-conjugated monoclonal antibodies was as described previously (Table I).

TABLE I

Membrane Perturbation of Peripheral T Cells with OKT3 Antibody and Horse α-Mouse IgG Induces Expression of Activation Antigens Defined by OKIa1, OKT9, and OKT10

OKT3	Horse α-mouse IgG	FITC Monoclonal antibody	Isotype	Percent of fluorescent cells*
−	−	OKIa1	IgG_{2a}	7, 10, 9, 5
+	−	OKIa1		9, 12, 11, 6
+	+	OKIa1		87, 95, 94, 91
−	−	OKT9	IgG_1	5, 4, 4, 4
+	−	OKT9		15, 12, 12, 11
+	+	OKT9		85, 93, 94, 95
−	−	OKT10	IgG_1	19, 21, 10, 23
+	−	OKT10		19, 20, 11, 15
+	+	OKT10		58, 61, 64, 59
−	−	OKT6	IgG_1	3, 1, 1, 1
+	−	OKT6		3, 1, 3, 1
+	+	OKT6		3, 1, 4, 1
−	−	OKT4	IgG_{2b}	64, 64, 62, 60
+	−	OKT4		67, 64, 62, 61
+	+	OKT4		67, 65, 65, 62
−	−	OKT8	IgG_{2a}	26, 37, 40, 36
+	−	OKT8		25, 36, 39, 40
+	+	OKT8		29, 36, 38, 40

* Results obtained from four separate experiments with different donors.

The presence of $OKIa1^+$ cells (5–10%) probably represents a minor contamination of the T cells with E^- cells, most of which are $OKIa1^+$; $OKT9^+$ T cells (4–5%) probably represent background staining; and $OKT10^+$ cells (19–23%) can be attributed to minor contamination by some E rosetting cells that may not represent classical T cells (26) and by E^- cells, some of which express the OKT10 antigen (27). Preincubation of peripheral T cells with OKT3 had only a minimal effect on OKIa1 staining (6–12%), caused a modest increase in OKT9 staining (11–15%), and had no effect on OKT10 staining (11–20%). Most strikingly, preincubation with OKT3 and horse α-mouse IgG resulted in marked increases of both fluorescent intensity and percentage of cells stained with OKIa1 (87–95%), OKT9 (85–95%), and OKT10 (58–64%) (Fig. 1 and Table I).

Similar results were obtained using W6/32, a monoclonal antibody to HLA framework, as the first antibody. In a representative experiment, comparing the percentage cells stained with first antibody alone and first antibody plus horse α-mouse IgG, OKT6 remained unchanged (2% and 3% respectively), whereas 69OKIa1 (4% and 43% respectively), OKT9 (1% and 70% respectively), and OKT10 (22% and 60% respectively) all showed increases in the percentage of stained cells and fluorescent intensity. This established that the membrane changes leading to display of activation antigens were not uniquely initiated by cross-linking of special T cell antigens.

One artifact that could produce data such as ours would be the binding of the fluoresceinated monoclonal antibodies to free α-mouse IgG sites on the horse α-mouse IgG bound to the first antibody on the cell surface. We tested this

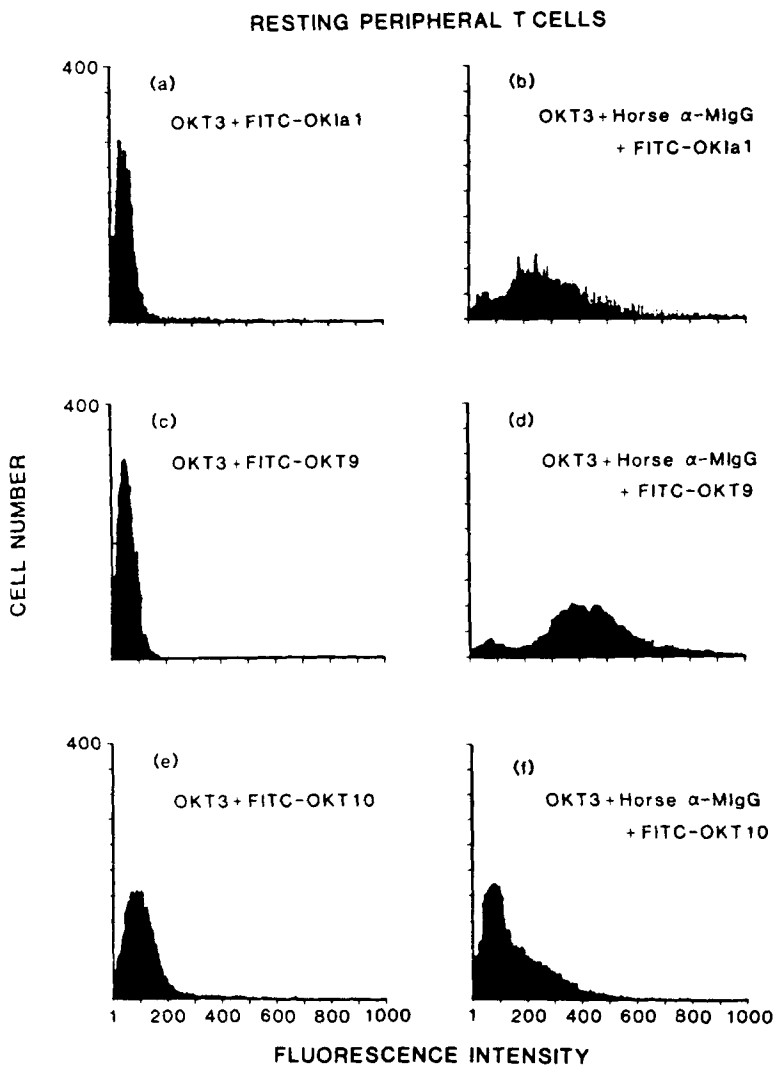

FIGURE 1. Fluorescence histograms of the distribution of activation antigens on resting peripheral T cells following membrane perturbation by cross-linking with OKT3 and horse α-mouse IgG. Cells were treated with: (*a*) OKT3 + FITC-OKIa1; (*b*) OKT3 + horse α-mouse IgG and FITC-OKIa1; (*c*) OKT3 + FITC-OKT9; (*d*) OKT3 + horse α-mouse IgG and FITC-OKT9; (*e*) OKT3 + FITC-OKT10; (*f*) OKT3 + horse α-mouse IgG and FITC-OKT10. The intensity of staining with FITC-OKIa1, FITC-OKT9, and FITC-OKT10 increased when the cells were pretreated with OKT3 and horse α-mouse IgG.

possibility by adding varying amounts of unlabeled mouse IgG and, as shown in Table II, 10 μg of unlabeled mouse IgG was sufficient to prevent binding of FITC-mouse IgG; our experiments used 50 μg of this preparation of mouse IgG, a dose that effectively occupied all the available sites. Furthermore, binding of FITC-OKT6, which is negative on resting peripheral T cells, remained negative after coating the cells with OKT3 and horse α-mouse IgG. In addition, no increase was observed in the number of $OKT4^+$ or $OKT8^+$ cells following

TABLE II

Inhibition of Fluoresceinated Mouse IgG Binding to Free Sites on Horse α-Mouse IgG by Nonfluoresceinated Mouse IgG

OKT3	Horse anti-mouse IgG	Nonfluoresceinated mouse IgG*	FITC mouse IgG‡	Percent of fluorescent cells§
+	+	—	+	92, 94
+	+	5	+	15, 11
+	+	10	+	3, 3
+	+	25	+	3, 3
+	+	50	+	2, 1
+	+	100	+	1, 2
+	−	—	+	1, 2
+	FITC α-MIgG	50	−	97, 97

* $\mu g/10^6$ cells.
‡ 5 $\mu g/10^6$ cells.
§ Individual results from two experiments.

treatment with OKT3 antibody and horse α-mouse IgG (Table I).

Despite these various controls we directed further experiments at identifying the newly expressed activation antigens by techniques that were independent of antibody binding, as detected by flow cytometry and immunofluorescence. OKT9 recognizes the transferrin receptor, albeit at a site distinct from the transferrin binding site (28), whereas 42/6 binds in the region of the binding size in that it competes with ^{125}I-transferrin in binding experiments (22). We found that control OKT9$^+$ and 42/6$^+$ cells, Raji cells, and 3-d PHA-activated T cells, bound ^{125}I-transferrin; with 95,000 cpm (100 ng ^{125}I-transferrin) input Raji cells bound 15,000 cpm (1,500 cpm in the presence of excess unlabeled transferrin) and 3-d PHA cells bound 6,000 cpm (1,400 cpm with excess unlabeled transferrin). In contrast, resting peripheral T cells treated with OKT3 plus horse α-mouse IgG, although OKT9$^+$ (see above), did not bind ^{125}I-transferrin and did not bind 42/6 (Fig. 2). We conclude that although membrane perturbation caused the exposure of some epitopes of the transferrin receptor molecule (as detected by OKT9), the actual transferrin receptor site (as detected by 42/6 and ^{125}I-transferrin) remained unexposed. It is of interest that two apparently normal individuals in our laboratories who donate blood were found to periodically have a high number of OKT9$^+$ peripheral T cells and these did not bind ^{125}I-transferrin.

The interleukin-2 (IL-2) receptor, recognized by the monoclonal antibody anti-Tac (23), also appears on T cells following activation. With membrane perturbation of resting T cells with OKT3 plus horse α-mouse IgG anti-Tac binding rose from 5% to 88%. The capacity of these cells to adsorb IL-2 was similar to that of 3-d PHA-activated cells (Fig. 3) and was markedly increased over that of resting T cells. In this instance it appears that membrane perturbation of resting T cells caused the exposure of the IL-2 binding region of this activation antigen.

Our interpretation of these various data was that cross-linking of cell surface bound monoclonal antibody by horse α-mouse IgG actually induced the cell surface display of the "activation" antigens. Procedures were carried out in the

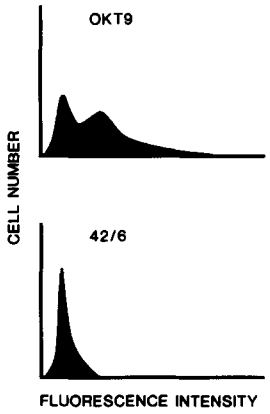

FIGURE 2. Fluorescence histograms of peripheral T cells treated with OKT3 + horse α-mouse IgG and then stained with FITC-OKT9 or 42/6, monoclonal antibodies to distinct epitopes on the transferrin receptor. OKT9 binding alone became positive (61%), whereas the epitope recognized by 42/6, which is situated in the region of the transferrin binding site, was not expressed (16%).

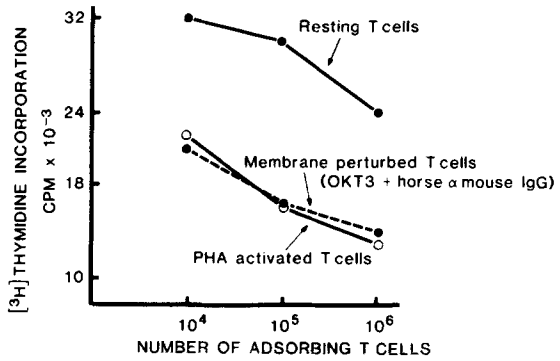

FIGURE 3. [^{3}H]thymidine uptake of late PHA-stimulated cells after addition of standard aliquots of IL-2 adsorbed by resting T cells, PHA-activated T cells and T cells with membranes perturbed by OKT3 plus horse α-mouse IgG. PHA-activated T cells and membrane-perturbed resting T cells adsorbed similar amounts of IL-2; the residual IL-2 activity after adsorption by these cells was far less than that left after adsorption by resting peripheral T cells.

presence of 0.01% sodium azide at 4°C, making it unlikely that these antigens could have been synthesized de novo during the time of the experiment. It seems likely that these antigens existed in or beneath the membrane in a form not detectable on the surface by monoclonal antibodies until membrane rearrangements, induced by the binding of the first monoclonal antibody and the horse α-mouse IgG, caused them to be revealed.

Expression of activation antigens on cells following membrane perturbations was restricted to those cells bearing antigen bound by the first monoclonal antibody. When OKT4 or OKT8 (reactive with ≃65% and 35% of the cells, respectively) was used to precoat cells, only the proportion of cells bearing the OKT4 or OKT8 antigen expressed the activation antigens after addition of horse-α mouse IgG (Fig. 4 and Table III). By contrast, perturbation of the cell surface of peripheral T cells with OKT3 and horse α-mouse IgG had no effect

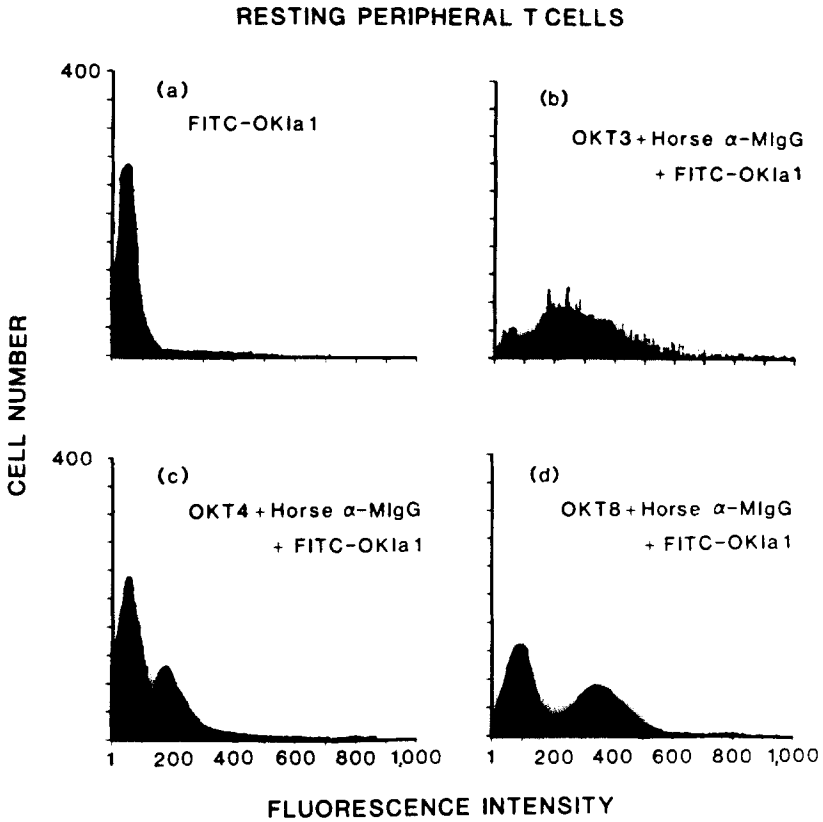

FIGURE 4. Fluorescence histograms of OKIa1 on peripheral E^+ cells before and after cross-linking of OKT3, OKT4 or OKT8 antigen with their respective antibodies and horse α-mouse IgG. Cells treated with: (*a*) FITC-OKIa1; (*b*) OKT3 + horse α-mouse IgG and FITC-OKIa1; (*c*) OKT4 + horse α-mouse IgG and FITC-OKIa1; (*d*) OKT8 + horse α-mouse IgG and FITC-OKIa1. OKT3 + horse α-mouse IgG induced Ia expression on most cells, whereas OKT4 or OKT8 + horse α-mouse IgG induced Ia expression only on subsets of T cells.

on the expression of OKT6, OKT4, or OKT8 (Table I), implying that these antigens represent stable markers of a differentiative stage of the T cell, and that they are not induced on cells lacking them by simple membrane rearrangements. Furthermore, display of activation antigens on $OKT4^+$ cells by membrane rearrangement was not accompanied by a similar display on $OKT8^+$ cells in the culture, and vice-versa, as determined by double staining (data not shown); thus, there was no evidence for extension of these phenomena by extracellular mediators.

We further studied these phenomena on human thymocytes. In this instance, binding of OKIa1 or OKT9 was low when cells were preincubated with OKT6 in the absence of horse α-mouse IgG (6–8% and 9–15% respectively); OKT10 binding to thymocytes was high as expected (95–97%), since OKT10 has been previously demonstrated on thymocytes (11). When thymocytes were preincubated with OKT6 and house α-mouse IgG, the fluorescence intensity and percentage of cells staining with FITC-OKIa1 (51–68%) and OKT9 (76–84%)

TABLE III

Membrane Perturbation of Peripheral T Cells with OKT4 or OKT8 Antibodies and Horse α-Mouse IgG Induces Expression of Activation Antigens Defined by OKT9 and OKIa1

	Horse α-mouse IgG	FITC monoclonal antibody	Percent of fluorescent cells*
OKT4			
−	−	OKT4	53, 60, 65
−	−	OKT9	1, 3, 3
+	−	OKT9	4, 4, 4
+	+	OKT9	36, 45, 55
−	−	OKIa1	3, 3, 3
+	−	OKIa1	4, 3, 4
+	+	OKIa1	36, 52, 49
−	−	OKT6	1, 1, 1
+	−	OKT6	1, 1, 1
+	+	OKT6	1, 3, 2
OKT8			
−	−	OKT8	35, 32, 34
−	−	OKT9	2, 1, 1
+	−	OKT9	2, 2, 1
+	+	OKT9	39, 31, 34
−	−	OKIa1	3, 3, 3
+	−	OKIa1	4, 3, 4
+	+	OKIa1	35, 30, 36
−	−	OKT6	1, 1, 1
+	−	OKT6	1, 2, 1
+	+	OKT6	3, 2, 1

* Results obtained from three separate experiments with different donors.

was markedly increased (Fig. 5 and Table IV), whereas staining with OKT10 remained unchanged (97–98%).

These results suggest a novel control mechanism for the display of certain cell membrane molecules; apparently these are present preformed within or beneath the cell membrane and are exposed on the cell surface by membrane perturbations. It will be important to test this hypothesis further by direct biochemical studies to determine whether these molecules reside within or beneath the cell membrane of resting T cells. It will also be important to determine whether the natural expression of these molecules is simply dependent on their reorientation in the membrane or whether chemical modifications involving protein or sugar changes or processes such as phosphorylation or acetylation are necessary for their expression. The presence of preformed molecules within the membrane could constitute a reservoir for molecules with functions that are required rapidly by the cell in response to stimuli altering its basal state. In general, the nature of these functions is unknown although OKT9 is known to react with the transferrin receptor that is needed for iron uptake in replicating cells (10) and anti-Tac reacts with the IL-2 receptor (23). It is interesting that antigens universally expressed on some cell types (for example, the Ia antigen on B cells and some monocytes and the OKT10 antigen on null cells and thymocytes) fall into the category of activation antigens on peripheral T cells.

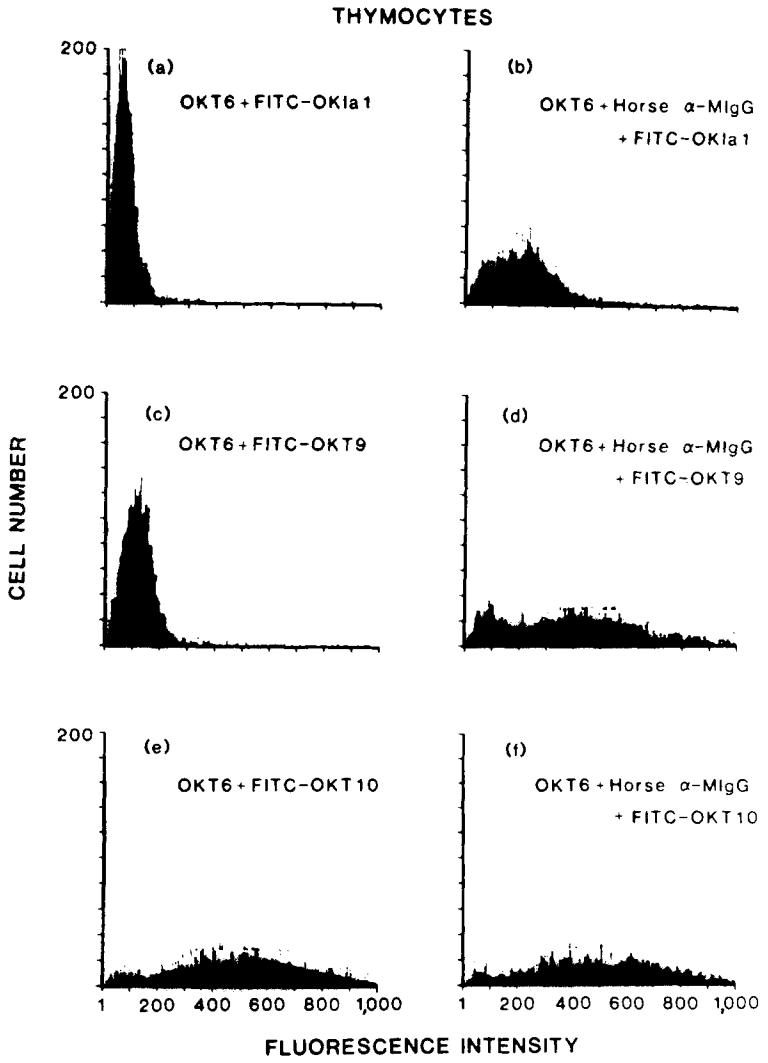

FIGURE 5. Fluorescence histograms of the distribution of activation antigens on thymocytes following membrane perturbation by cross-linking with OKT6 and horse α-mouse immunoglobulin. Cells were treated with: (*a*) OKT6 + FITC-OKIa1; (*b*) OKT6 + horse α-mouse IgG and FITC-OKIa1; (*c*) OKT6 + FITC-OKT9; (*d*) OKT6 + horse α-mouse IgG and FITC-OKT9; (*e*) OKT6 + FITC-OKT10; (*f*) OKT6 + horse α-mouse IgG and FITC-OKT10. The staining intensity of OKIa1 and OKT9 increased when the cells were treated with OKT6 and horse α-mouse IgG before FITC-OKIa1 or FITC-OKT9, whereas the fluorescence staining intensity with OKT10 was high initially and remained unchanged.

Many double-staining protocols employ initial indirect immunofluorescence (resulting in cross-linking by α-mouse IgG) followed by direct immunofluorescence; awareness that the first steps may actually induce expression of the antigen recognized by the direct reagent is essential for the proper design and interpretation of such experiments.

TABLE IV

Membrane Perturbation of Thymocytes with OKT6 Antibody and Horse α-Mouse IgG Induces Expression of Activation Antigens Defined by OKIa1 and OKT9

OKT6	Horse α-mouse IgG	FITC monoclonal antibody	Isotype	Percent of fluorescent cells*
−	−	OKIa1		6, 3, 4, 3
+	−	OKIa1	IgG_{2a}	8, 7, 7, 6
+	+	OKIa1		62, 63, 51, 68
−	−	OKT9		4, 4, 4, 5
+	−	OKT9	IgG_1	10, 12, 9, 15
+	+	OKT9		78, 76, 84, 78
−	−	OKT10		98, 96, 98, 96
+	−	OKT10	IgG_1	97, 95, 96, 97
+	+	OKT10		97, 97, 98, 97

* Results obtained from four separate experiments with different donors.

Summary

Three human lymphocyte antigens recognized by monoclonal antibodies OKIa1, OKT9, and OKT10 were found to be minimally represented on resting peripheral T cells (all three) and thymocytes (OKIa1 and OKT9). These antigens, which are present on "activated" T cells, were promptly displayed on "resting" T cells or thymocytes following cross-linking of surface-bound monoclonal antibody by horse α-mouse IgG. These experiments suggested that membrane perturbations may induce the expression of certain antigens that are normally present in an unexpressed form in resting cells.

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