HUMAN T LYMPHOCYTE SUBPOPULATIONS DEFINED BY Fc RECEPTORS AND MONOCLONAL ANTIBODIES

A Comparison*

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Several approaches have provided evidence for the existence of distinct T lymphocyte subsets in man as defined by cell-surface markers. To a large extent, these studies have employed either differential Fc-receptor binding, or antisera reactive selectively with subpopulations of T cells. With the former method, it was shown that two human T cell subsets, T cells bearing receptors for the Fc portion of IgG ($T\gamma$) and T cells bearing receptors for the Fc portion of IgM ($T\mu$), could be isolated via surface receptors capable of binding the Fc portion of IgG or IgM, respectively (1, 2). Approximately 60–70% of T lymphocytes in peripheral blood were defined as $T\mu$, whereas a smaller portion representing <20% were $T\gamma$ cells. Functionally, $T\gamma$ and $T\mu$ populations were distinct with respect to phytohemagglutinin responsiveness and capacity to effect help or suppression of B cell-immunoglobulin production in a pokeweed-mitogen-driven system (3, 4).

Hetero- and autoimmune antisera and hybridomas that secrete monoclonal antibodies directed at subsets of human T cells have provided an alternative method for their identification (5–16). 20% of peripheral T cells were found to be reactive with TH_2 heteroantisera (TH_2^+) or a monoclonal antibody termed OKT5 ($OKT5^+$) and both defined the cytotoxic/suppressor populations (6, 7, 17). The remaining 80% of T cells were unreactive with both TH_2 and OKT5 antisera and defined as TH_2^- ($OKT5^-$). A second monoclonal antibody, OKT4, reacted with 60% of the total T cell population and was restricted in its reactivity to the TH_2^- ($OKT5^-$) T cell subpopulation (12–16). Only the $OKT4^+$ T cell population responded with proliferation to soluble antigen (12). More importantly, this population was the helper population because it was required for the optimal development of the TH_2^+ ($OKT5^+$) cytotoxic cell in cell-mediated lympholysis, induction of B cell differentiation, proliferation and immunoglobulin synthesis in a pokeweed-mitogen-driven system, and production of helper factors (13–16). Thus, the $OKT4^+$ and $OKT5^+$ T cells belonged to reciprocal subsets, and defined the human inducer (helper), and suppressor, T cell subpopulations, respectively. Because of the importance of relating T lymphocyte

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subsets defined by both monoclonal antibodies and Fc receptors to one another, these populations were isolated and compared.

Materials and Methods

Separation of T Cell Subpopulations by Differential Fc-Receptor Binding. Separation of unfractionated T cells, Tγ-enriched, Tγ-depleted, Tμ, and T cells depleted of Tγ and Tμ subpopulations (T_{Null}) populations was performed as described in detail elsewhere (4). Purity of Tγ and Tμ subpopulations was >96% by analysis of Fc-receptor rosetting.

Separation of OKT4- and OKT5-reactive Populations. Production, growth, and characterization of the hybridoma-secreted monoclonal antibodies OKT4 and OKT5 were the subject of prior reports (12, 17). With fluorescence-activated cell sorter (FACS-I) (Becton, Dickinson, FACS Systems, Mountain View, Calif.), T cells were separated into $OKT4^+$ and $OKT5^+$ populations as previously described, and then cultured in 20% fetal calf serum overnight (12). Purity and viability of the FACS-separated T cell subsets were both >95%. Enumeration of Tγ and Tμ cells within the unfractionated T cell population, the unfractionated T cell population incubated with monoclonal antibody and fluorescein-conjugated goat anti-mouse IgG (G/M FITC) (antibody treated [Rx]), and FACS-separated $OKT4^+$ and $OKT5^+$ subsets was determined as previously described (2).

Surface Markers on Separated Lymphocyte Subpopulations. T cell subpopulations obtained by Fc-receptor rosetting were analyzed after 24 h with monoclonal antibodies. In addition to OKT4 and OKT5, other monoclonal antibodies were utilized; one, termed OKT3, was previously shown to react with 100% of peripheral T cells and no other lymphoid elements (13, 14); a second, termed OKI1, was shown to react with the bimolecular glycoprotein complex of 29,000- and 34,000-dalton subunits of the Ia molecule in man (14); and a third, termed OKM1, was utilized to define monocytes because it reacts selectively with virtually all adherent macrophages required for antigen presentation in T cell proliferation and a population of nonadherent monocytes.[1] Indirect immunofluorescence was performed with monoclonal antibodies at a 1:250 dilution as described in prior reports. The negative control ascites was OKT6, a monoclonal antibody reactive selectively with human thymocytes but not peripheral lymphocytes (18).

Results and Discussion

Unfractionated T cells and Tγ- and Tμ-enriched subpopulations were analyzed from a single individual by the use of monoclonal antibodies and indirect immunofluorescence on the FACS. As shown by the representative case in Fig. 1, ~95% of the unfractionated E-rosetting population was reactive with the OKT3 monoclonal antibody, whereas 65% were reactive with OKT4 and 20% were reactive with OKT5. The 5% of cells forming rosettes with sheep erythrocytes ($E\text{-}RFC^+$) unreactive with OKT3 were reactive with OKM1, a hybridoma antibody that defines an antigen present on monocytes. In addition, 3% of cells in the same unfractionated T cell population were specifically reactive with OKI1 defining the Ia antigen in man.

The Tμ population was similar to the unfractionated T cell population with respect to reactivity with OKT3, OKT4, and OKT5 (Fig. 1). However, unlike the unfractionated T cell population, OKI1- and OKM1-reactive cells had been depleted after the removal of the Tγ population. In contrast, the Tγ-enriched population contained only 10% T cells as defined by OKT3 monoclonal antibody. Within this T cell fraction, there was no selectivity for either the $OKT4^+$ inducer or $OKT5^+$ suppressor T cell subset, because both were represented in the same ratio as found in the unseparated T cell population. More importantly, it was found that the major portion of lymphocytes in the Tγ-enriched population were mononuclear cells that bore the

[1] Breard, J. M., E. L. Reinherz, P. C. Kung, G. Goldstein, and S. F. Schlossman. A monoclonal antibody reactive with human peripheral blood monocytes. *J. Immunol.* In press.

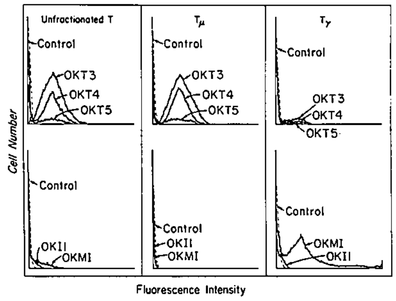

FIG. 1. Reactivity of unfractionated T cells and T cell subsets defined by Fc receptors with monoclonal antibodies utilizing indirect immunofluorescence on FACS. See Results and Discussion for additional information.

OKM1 marker. In addition, it should be noted that the Tγ cells were predominantly Ia^- as determined by reactivity with OKI1.

Table I provides results from two of five additional experiments in which Tγ-enriched, Tγ-depleted, Tμ, T_{Null}, and unfractionated T populations were reacted with these monoclonal antibodies. The data clearly demonstrate the reactivities of Tγ-depleted, Tμ, and T_{Null} subpopulations were virtually identical to the unfractionated population. Again, the Tγ-enriched population was largely composed of cells that lacked reactivity with all of these T cell monoclonal antibodies and that shared reactivity with a monocyte-reactive monoclonal antibody, OKM1 (18).

Thus, the Tγ-enrichment procedure did not select for subsets of T cells as defined by these monoclonal antibodies. Three additional experiments gave similar results and showed that the Tγ-enriched population was heterogeneous because anywhere from 5 to 50% of cells were OKT3 reactive, 50 to 90% were OKM1 reactive, and 3 to 25% were OKI1 reactive. It should be noted that a prior study demonstrated the existence of $OKM1^+$ mononuclear cells that were predominantly Ia^-.[1]

To determine the relationship of separated $OKT4^+$ and $OKT5^+$ T cell subsets to the Tγ and Tμ lymphocyte subpopulations, unfractionated T cells or FACS-separated T cell subsets were rosetted with ox erythrocytes that were coated with either rabbit IgG or IgM antibodies. Subsequently, the percentages of Tγ and Tμ cells were enumerated. As shown in Table II, the unfractionated T cells, the unfractionated T cells treated with monoclonal antibody and G/M FITC (OKT4 Rx or OKT5 Rx), and $OKT4^+$ or $OKT5^+$ T cell subsets were all comparable in their percentage of Tγ-reactive cells. Thus, isolation of either $OKT4^+$ or $OKT5^+$ T cell subsets provided no significant enrichment for the Tγ population. However, Table II also shows that there was a partial enrichment of the Tμ-rosetting cells in the $OKT4^+$ subset. These latter results supported the notion that the population of T cells defined by the monoclonal antibody OKT4 and that defined by Tμ were in part overlapping. This was not surprising in light of prior functional studies which demonstrated that both Tμ and $OKT4^+$ T cell subsets contained the T inducer population for B cell-immunoglobulin production (4, 15).

Other than the partial enrichment of Tμ cells within the $OKT4^+$ subset, there was generally little correlation in the cell subpopulations defined by monoclonal antibodies and those defined by Fc receptors. Moreover, the Tγ-depleted, Tμ, and T_{Null} subpopulations defined by Fc receptors were virtually indistinguishable from the unfraction-

TABLE I

Reactivity of T Cell Subsets Defined by Fc Receptors with Monoclonal Antibodies

Experimental number	Monoclonal antibody	Unfractionated T	Tγ enriched	Tγ depleted	Tμ	T_{Null}
				%		
1	OKT3	93	14	93	95	89
	OKT4	65	8	64	69	58
	OKT5	25	7	25	25	25
	OKI1	5	3	2	1	3
	OKM1	6	50	3	1	8
2	OKT3	84	15	90	95	85
	OKT4	61	10	68	73	55
	OKT5	22	7	25	18	25
	OKI1	4	8	1	1	0
	OKM1	18	88	3	2	7

ated T cell population in reactivity with OKT3, OKT4, and OKT5. The single exception was the Tγ-enriched population, which had few T cells as defined by OKT3 and had a large number of $OKM1^+$ cells. In further support of these latter findings were the previous functional studies that demonstrated that the unfractionated T cell population and the Tμ subpopulation were similar. Thus, both unfractionated T cells and Tμ cells could provide help for B cell-immunoglobulin secretion, effect cell-mediated lympholysis, and be induced by concanavalin A (Con A) to suppress (4, 19, 20). In contrast, it has been shown that the $OKT4^+$ subset alone could provide help in T-T and T-B interactions, whereas the $OKT5^+$ subset alone could effect cell-mediated lympholysis and was induced by Con A to suppress (12, 15, 17).

The Tγ population of cells was distinct from the unfractionated T cells and all other subsets of T cells isolated with Fc rosetting by virtue of its small percentage (15%) that showed reactivity with the T cell-specific monoclonal antibodies and its large percentage (50–88%) that showed reactivity with the OKM1 monoclonal-antibody defining monocytes. Although both Tγ and $OKT5^+$ subpopulations can be induced to suppress with Con A, there are functional differences of note between these populations (17, 19, 20). Specifically, $OKT5^+$ cells proliferate in mixed lymphocyte cultures and effect cell-mediated-lympholysis, whereas Tγ cells do not. Conversely, Tγ cells effect natural-killer activity and antibody-dependent cellular-cytotoxic function that are not properties of unactivated $OKT5^+$ cells (17, 21, 22).

Our results indicate that circulating lymphoid cells identified as Tγ cells (i.e., nonadherent $E\text{-}RFC^+$ and Fc-receptor-portion of IgG positive) include at least two cell types: (*a*) a small population reactive with OKT3, and (*b*) a large population reactive with OKM1. Those cells expressing T cell antigens recognized by our monoclonal antibodies averaged only 22% of the Tγ preparations in these six experiments. The lack of reactivity of most Tγ cells with three monoclonal antibodies thought to define all peripheral T cells and a monoclonal antibody specific for human thymocytes (OKT6) suggests that the majority that displayed distinct morphologic and histochemical features (23) are neither mature nor immature cells of T lineage (12–17, 18). Moreover, the reactivity with the OKM1 monoclonal antibody that defined an antigen shared by monocytes and granulocytes implies that they may rather be of monocyte-myeloid lineage. We suggest that these cells are the natural-killer cells.

TABLE II

Capacity of Monoclonal-Antibody-separated T Cell Subsets to Rosette with Ox Erythrocytes Coated with IgG and IgM

Cell Population	Experiment 1		Experiment 2	
	Fcγ‡	Fcμ§	Fcγ‡	Fcμ§
		%		
E-RFC⁺	4.0	57	7	58
OKT4 Rx*	5.5	48	7	61
OKT4⁺	2.5	88	1	90
OKT5 Rx*	6.5	56	6.5	58
OKT5⁺	2.5	34	7	50

* Control T cell populations were reacted with either OKT4 or OKT5 and G/M FITC but not sorted on FACS.

Summary

Human T cell subpopulations have been defined on the basis of differential expression of either Fc receptors or specific cell-surface antigens. In this study, we utilized a series of monoclonal antibodies reactive with T cells, monocytes, and Ia antigens to characterize isolated subpopulations of T cells bearing receptors for the Fc portion of IgG (Tγ) and subpopulations of T cells bearing receptors for the Fc portion of IgM Tμ. The results showed that the Tμ population contained both inducer ($OKT4^+$) and cytotoxic/suppressor ($OKT5^+$) populations and was similar to the unfractionated T cell population, whereas the Tγ subset contained few T lymphocytes ($OKT3^+$) and was not enriched for either T cell subset defined by these monoclonal antibodies. Rather, the Tγ population was comprised largely of Ia^- cells possessing a monocyte antigen ($OKM1^+$). In reciprocal studies, it was found that both isolated $OKT4^+$ and $OKT5^+$ T cell subsets contained few Tγ cells, whereas both subsets were mainly comprised of Tμ cells. We conclude that there is little correlation between T cell subsets defined by these monoclonal antibodies and those defined by Fc receptors.

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