Ia DETERMINANTS ON HUMAN T-CELL SUBSETS DEFINED BY MONOCLONAL ANTIBODY

Activation Stimuli Required for Expression*

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Immunoregulatory activities are mediated, in part, by intercellular signals involving products of the I-region (Ia antigen) of the major histocompatibility complex in several species. Human Ia-like antigens were first defined by alloantisera and subsequently by heteroantisera. In man, Ia determinants are expressed on the surface of B cells, monocytes, and a subset of Null cells (1–4). In contrast, Ia antigens are not readily detectable on T cells or are, at best, expressed on an extremely small percentage of resting T lymphocytes (4–6). Perhaps of greater importance is the recent finding by several laboratories that activated human T cells express Ia antigens (5, 6). Several observations point to the fact that these Ia antigens are intrinsic to the T cells and are not passively absorbed: (*a*) the demonstration of Ia^+ T-cell leukemias; (*b*) the finding that Ia antigens expressed by alloactivated T cells in mixed lymphocyte culture (MLC)[1] are of the responder and not the stimulator DR specificity; and (*c*) activated T cells biochemically synthesize Ia antigens de novo (4–8).

The present study was directed at further characterization of Ia antigens which appear on human T cells after activation and the stimuli required for their expression. To avoid the complexity of alloantisera and heteroantibodies, we utilized several monoclonal antibodies including one reactive with the Ia antigen framework for characterization of Ia-like antigens, a second monoclonal antibody specific for the entire T-cell population, and a third directed at the T-cell inducer (helper) subset. In the results to be reported below, it will be shown that the total T-cell population and individual subsets of T cells can be activated to express Ia antigens when stimulated by alloantigens or mitogens. In contrast, only the inducer T-cell population expresses Ia antigen after activation by tetanus toxoid.

These studies support the notion that T cells which do not proliferate to soluble

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[1] *Abbreviations used in this paper:* Con A, concanavalin A; DTT, dithiothreitol; E, sheep erythrocyte rosette; FACS, fluorescence-activated cell sorter; G/M FITC, fluorescein-conjugated goat-anti-mouse IgG; MLC, mixed lymphocyte culture; NP40, nonidet P40; PHA, phytohemagglutinin; PMSF, phenylmethylsulfonyl; STI, soybean trypsin inhibitor.

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antigen do not express Ia-like molecules after exposure to antigen. In addition, pretreatment of T cells with anti-Ia and complement did not eliminate the appearance of Ia antigens on T cells after activation, therefore, suggesting that activation induces the expression of Ia antigen on Ia^- T cells and this expression is not the consequence of clonal expansion of a small subset of Ia^+ T cells.

Materials and Methods

Production of Monoclonal Antibodies

IMMUNIZATION AND SOMATIC CELL HYBRIDIZATION. 8-wk-old female mice, strains BALB/cJ or CAF_1 (The Jackson Laboratories, Bar Harbor, Maine), were immunized intraperitoneally with 2×10^7 sheep erythrocyte (E) rosette-purified peripheral T cells in phosphate-buffered saline at 14-d intervals. 4 d after the third immunization, the spleens were removed and a single cell suspension was prepared. Cell fusion was carried out according to the procedure developed by Kohler and Milstein (9). For each fusion, 1×10^8 splenocytes were fused with 35% polyethylene glycol (PEG), 5% dimethyl sulfoxide, and RPMI-1640 (Grand Island Biological Co., Grand Island, New York) with 2×10^7 P3X63Ag8U1 myeloma cells.

SELECTION AND GROWTH OF HYBRIDOMAS. After cell fusion, cells were cultured in hypoxanthine, aminopterin, and thymidine medium at 37°C with 5% CO_2 in a humid atmosphere. Several weeks later, 40–100 μl of supernate from cultures containing hybridomas were tested on 10^6 peripheral lymphocytes separated into E rosette positive (E^+) and E rosette negative (E^-) populations which were prepared from the peripheral blood of healthy human donors as previously described (10). Detection of mouse hybridoma antibodies to these cells was determined by indirect immunofluorescence. Cells incubated with cell supernates were stained with a fluorescein goat anti-mouse IgG (G/M FITC) (Meloy Laboratories Inc., Springfield, Va.) and the fluorescent cells were subsequently analyzed on the Cytofluorograf FC200/4800A (Ortho Instruments, Westwood, Mass.) as described below. Hybridoma cultures containing antibodies reacting selectively with E^+ and E^- lymphocytes were selected, cloned, and recloned by limiting dilution methods in the presence of feeder cells. Subsequently, the clones were transferred intraperitoneally by injecting 1×10^7 cells of the given clone into CAF_1 mice primed with pristane (Aldrich Chemical Co. Inc., Milwaukee, Wis.). The malignant ascites from these mice were then used to characterize lymphocytes as described below. The monoclonal antibody product of one of these clones which reacted specifically with E^- cells was termed OKI1. The OKI1 hybridoma antibody was demonstrated to be of the subclass by the specificity of its staining with fluorescein-labeled goat anti-mouse IgG_2 (Meloy Laboratories Inc.) and its failure to be stained by fluorescein-labeled antibodies against other subclasses of mouse immunoglobulins.

Fractionation of Lymphocytes. Human peripheral blood mononuclear cells were isolated from healthy volunteer donors by Ficoll-Hypaque density gradient centrifugation (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway N.J.). Unfractionated mononuclear cells were separated into surface Ig^+ (B) and Ig^- (T plus Null) populations by Sephadex G-200 anti-$F(ab')_2$ affinity chromatography as previously described (11). T cells were recovered by E rosetting the Ig^- population with 5% sheep erythrocytes (Microbiological Associates, Walkersville, Md.). The rosetted mixture was layered over Ficoll-Hypaque and the recovered E^+ pellet treated with 0.155 M NH_4Cl. In addition, the nonrosetting Ig^- (Null cell) population was harvested from the Ficoll interface. The surface Ig^+ (B) population was obtained from the Sephadex G-200 column after elution with normal gamma globulin as previously described (11). Normal human macrophages were obtained from the mononuclear population by adherence techniques as previously reported (12, 13).

In experiments designed to obtain highly purified T-cell populations, several alternative isolation techniques were employed in addition to those described above. In one approach, T cells were E rosetted with sheep erythrocytes as above, reacted with OKT3 and G/M FITC, and the total T cells sorted with OKT3 on the fluorescence-activated cell sorter (FACS-I) (Becton, Dickinson & Co., Oxnard, Calif.) as described below. The OKT3 monoclonal antibody was previously shown to selectively react with the entire human peripheral T-cell subpopulation but not B cells, Null cells, or macrophages (14). In a second approach, T cells were first

obtained by passage through nylon wool and subsequent E rosetting. This population of nylon-passed E rosetted cells was then utilized either directly or after separation on the FACS with OKT4 or OKT3 monoclonal antibody. OKT4 was previously shown to be specific for the human inducer (helper) subset (12).

Isolation of the Cell Surface Antigen Defined by OKI1. 5×10^8 cells from the B lymphoblastoid cell line Laz 388 were washed and resuspended in 100 ml of methionine-free minimal essential medium with Earle's Salts (SMEM). Cells were incubated for 16 h at 37°C with 1 mCi of [^{35}S]methionine (New England Nuclear, Boston, Mass.) and harvested by centrifugation. Pellets were resuspended in 0.01 M Tris buffer supplemented with phenylmethylsulfonyl fluoride (PMSF), soybean trypsin inhibitor (STI), and dithiothreitol (DTT). After hypotonic lysis, the sample was pelleted at 1,500 *g*. Supernates were resuspended in 2% nonidet P40 (NP40) in 0.01 M Tris at pH 7.8 supplemented with PMSF, STI, and DTT and stirred on ice for 45 min. Insoluble material was pelleted at 13,000 *g* for 20 min and the supernates collected and counted.

The soluble ^{35}S methionine-labeled membrane extracts (50×10^6 cpm) were applied to a 2-ml column of Sepharose 4B-bound lentil lectin (Pharmacia Fine Chemicals, Div. of Pharmacia Inc.) at 4°C. The column was washed with 500 ml of lectin buffer (0.001 M Tris HCl pH 7.4, 0.14 M NaCl, 0.1 mM Mn Cl_2, 0.1 mM $CaCl_2$, and 0.2% NP40). Bound glycoproteins were then eluted with a 3% solution of α-methyl-D-mannoside in lectin buffer. Peak tubes were pooled and evenly distributed between Sepharose-protein A-bound OKI1 and Sepharose-protein A-bound control myeloma ascites. Samples were incubated overnight at 4°C in a mechanical rotater and thoroughly washed with buffer containing NP40. 100 μl of sample buffer containing 10% glycerol, 5% mercaptoethanol, 3% sodium dodecyl sulfate (SDS), and 0.005% bromophenol blue in 0.125 M Tris HCl was added to each pelleted Sepharose-protein A bound sample before a 5-min incubation at 110°C. Samples were then loaded onto a 7–20% gradient polyacrylamide slab gel (7 × 11 in) (15). Electrophoresis was performed at 150 V for 18 h. Gels were stained, fixed, dehydrated, impregnated with 2,5 diphenyloxazole, and dried using the method of Bonner and Laskey (16). A sheet of Kodak X-Omat R film (Eastman Kodak Co., Rochester, N. Y.) was placed on the dried gel and exposed for 48 h at −70°C.

Cytofluorographic Analysis and Cell Separation. Cytofluorographic analysis of all cell populations was performed by indirect immunofluorescence with fluorescein-conjugated goat anti-mouse IgG (Meloy Laboratories Inc.) on a Cytofluorograf FC200/4800A (Ortho Instruments). In brief, 2×10^6 cells were treated with 0.15 ml of either of three monoclonal antibodies termed OKI1, OKT3 or OKT4, at a 1:1,000 dilution, incubated at 4°C for 30 min, and washed twice. The cells were then reacted with 0.15 ml of a 1:40 dilution of G/M FITC for 30 min at 4°C, centrifuged, and washed three times. These cells were then subsequently analyzed on the Cytofluorograf and the intensity of fluorescence per cell recorded on a pulse height analyzer. Background staining was obtained by substituting a 0.15-ml aliquot of 1:1,000 ascites from a BALB/c mouse injected intraperitoneally with a nonproducing clone.

In experiments designed to separate $OKT3^+$, $OKT4^+$, and $OKT4^-$ cells, 100×10^6 cells were labeled with 4 ml of a 1:1,000 dilution of monoclonal antibody and developed with G/M FITC. Utilizing the fluorescence-activated cell sorter (FACS), T cells were separated into hybridoma reactive and nonreactive populations as previously described (17). Post sort viability was >95% by trypan blue exclusion in all instances.

Analysis of FACS-separated T-Cell Subsets with OKI1. Unfractionated T cells obtained by affinity chromatography and E rosetting, nylon passage and E rosetting, and/or FACS separation with OKT3 monoclonal antibody and indirect immunofluorescence, as well as FACS-fractionated $OKT4^+$ and $OKT4^-$ T-cell subsets were placed in culture at 2×10^6 cells/ml in RPMI-1640 (Grand Island Biological Co.) containing 20% human AB serum, 1% penicillin-streptomycin, 200 mM L-glutamine, 25 mM Hepes buffer (Microbiological Associates), and 0.05% sodium bicarbonate. After variable time intervals (1–9 d) in 5% CO_2 humid atmosphere at 37°C with or without mitogen, antigen, or alloantigen as activation stimuli, 1-2 $\times 10^6$ cells of each population were reacted with OKI1 and stained with G/M FITC. Background staining was determined as described above.

T-Cell Stimulation by Alloantigens, Soluble Antigens, and Mitogens. T cells were stimulated with a B-lymphoblastoid line Laz 156 in MLC and with doses of phytohemagglutinin (PHA) and concanavalin A (Con A) ranging from 0.1 to 0.01 μg and 50 to 250 μg, respectively, as previously described (12). Stimulation with tetanus toxoid was performed by utilizing 10 μg/ml tetanus

toxoid (Massachusetts Department of Public Health Biological Laboratories, Boston, Mass.). 5% macrophages were added to all mitogen and soluble antigen stimulated populations at the initiation of cell cultures.

Complement-Mediated Lysis of T Cells. Complement-mediated lysis of MLC-activated or unactivated T cells with OKT3 and OKI1 were performed in microplate assay. 2×10^6 T cells were treated with 0.2 ml [^{51}Cr]sodium chromate (292 μCi/ml) (New England Nuclear) and incubated in a 37°C shaking water bath for 90 min. Cells were spun down, washed twice, and diluted to 2×10^5/ml. 20 μl of labeled target cells (4,000 cells) were added to microplate wells with 20 μl of hybridoma antibody for 1 h at 20°C at dilutions between 10^{-2} and 10^{-7} done in triplicate. Subsequently, 20 μl of fresh rabbit complement (1:10 dilution) was added to each well and incubated at 37°C for an additional hour. After incubation, 140 μl of media was added to each well and plates were spun for 5 min at 300 *g* and 100 μl of supernate was aspirated and counted on a gamma scintillation counter (Packard Instrument Co., Downers Grove, Ill.). Freeze-thaw and spontaneous release samples included complement and media. Specific complement mediated lysis was calculated using the following formula:

$$\text{Percent specific lysis} = \frac{\text{Exp.} - \text{SR}}{\text{FT} - \text{SR}} \times 100;$$

where Exp. = mean of the observed triplicate, SR = spontaneous release, and FT = freeze thaw.

For lysis of larger number of cells with antibody and complement, 20×10^6 T cells were spun down in 15-ml tubes (Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, Calif.), 1 ml of OKI1 at a 1:1,000 dilution added, resuspended, and incubated for 1 h at 20°C. Subsequently, 0.1 ml fresh rabbit complement was added, resuspended and incubated for an additional hour in a shaking water bath at 37°C. Cells were spun down, washed twice, brought up in RPMI-1640 supplemented with 20% human AB serum and placed in culture with or without the alloantigen Laz 156. At the end of 6 d, these cells were harvested and analyzed for expression of OKI1 antigen by indirect immunofluorescence.

Results

Characterization of OKI1 Antibody Reactivity on Normal Human Lymphoid Cells. Highly purified T, B, Null, and macrophage populations were prepared from a given individual's peripheral mononuclear population. Fig. 1 shows the fluorescence pattern of 10,000 cells obtained on the Cytofluorograf after reacting these populations with OKI1 at a 1:1,000 dilution and G/M FITC. As shown, the entire T-cell population obtained by affinity chromatography and E rosetting is unreactive. In contrast, the vast majority of B cells and macrophages in excess of 90% were specifically reactive with OKI1. In addition, ~20% of Null cells are also reactive. An identical pattern of reactivity was seen with lymphocyte subpopulations from 20 other normal individuals (data not shown).

Molecular Identification of the Antigen Defined by OKI1. The cellular expression of the antigen defined by OKI1 was virtually identical to that defined with previously reported alloantisera and heteroantisera defining Ia antigens (4). To determine the antigen identified by OKI1, an immunoprecipitation technique was employed utilizing a B lymphoblastoid line, Laz 388. As shown in Fig. 2, OKI1 specifically precipitates two major bands from this Ia antigen-bearing cell line. These included a broad band centered at 34,000 daltons and a sharper band at 29,000 daltons, which together define the components of the Ia antigen. A less intense band at 30,000 daltons is also seen. This has been previously observed utilizing heteroantisera specific for Ia and may represent a breakdown product or biological variant of the 34,000 dalton chain (18).

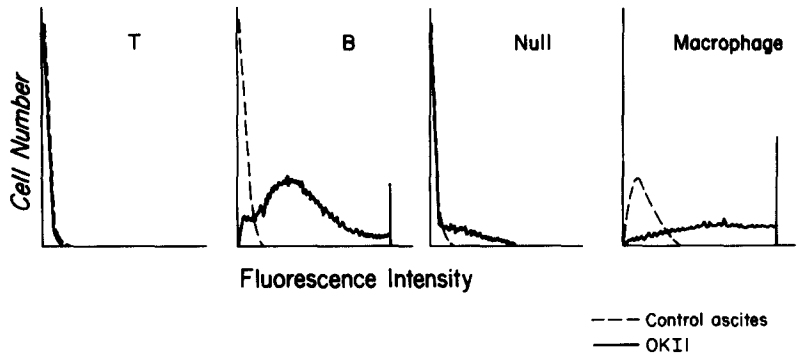

FIG. 1. The reactivity of OKI1 with human lymphocyte subpopulations by indirect immunofluorescence (solid line). As shown, the human T-cell population is unreactive with OKI1 (1:1,000 dilution). In contrast, the antigen defined by OKI1 is present on the vast majority of B cells and macrophages (>9,100 of 10,000 cells tested). In addition, OKI1 reacts with 20% of human Null cells (2,207 of 10,000 cells tested). Background fluorescence staining (dotted line) was obtained by incubating each population with a 1:1,000 dilution ascitic fluid from a mouse injected with a nonproducing clone.

Expression of Ia-like Antigens on Activated and Unactivated T-Cell Subsets. To explore in detail the possibility that human peripheral blood T cells bear Ia-like antigens, T cells were initially separated by several isolation techniques and the entire T-cell population or T-cell subset further purified by using monoclonal antibodies specific for the entire T-cell population or inducer T-cell subset. These T cells were then examined for expression of Ia-like antigens before or after activation using the above monoclonal anti-Ia antibody. As shown in Fig. 3, T cells selected by E rosetting and reactivity with OKT3 were entirely unreactive with OKI1 after in vitro culture for 6 d. Similarly, T cells selected by nylon adherence and E rosetting were likewise unreactive. Moreover, when these T cells were fractionated on the FACS into $OKT4^+$ and $OKT4^-$ T cell subsets, there was still no evidence of reactivity. Similar results were obtained when T cells were examined on days 1, 3, 4, and 9 in tissue culture. In contrast, as shown in Fig. 4, when T cells were stimulated by alloantigen in MLC, ~65–70% expressed OKI1 antigen on day 6. Greater than 98% of these MLC-activated lymphocytes were T cells as assessed by E rosette capacity and reactivity with OKT3 and indirect immunofluorescence (data not shown).

In addition to the absence of detectable surface Ia-like molecules on resting T cells by indirect immunofluorescence, the appearance of Ia-like molecules on MLC activated T cells was confirmed by complement lysis studies. $OKT3^+$ and complement were lytic for all T cells to a dilution of 10^{-6}. When this same T-cell population was treated with OKI1 and complement, there was no evidence of lysis at any dilution (Fig. 5 A). After alloactivation, however, it could be seen that OKI1 and OKT3 were now lytic for 75 and 100% of T cells, respectively (Fig. 5 B).

To determine whether Ia antigens appeared on all subsets of alloactivated T cells, T cells were fractionated into $OKT4^+$ and $OKT4^-$ populations and stimulated by alloantigen in MLC. As shown in Fig. 4, both $OKT4^+$ and $OKT4^-$ T cells expressed Ia antigen after activation. In addition, Table I shows that PHA or Con A activation of both $OKT4^+$ and $OKT4^-$ T-cell subsets also induced the appearance of Ia antigens on a fraction of T cells. However, it should be noted that these mitogens induced a

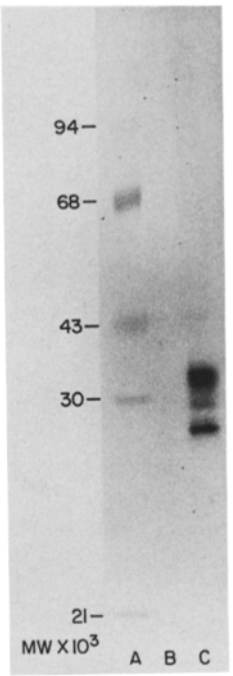

FIG. 2. Autoradiograph of OKI1 immune complex with ^{35}S-labeled B-cell line. Denatured chemically reduced samples were run on a large 7–20% gradient acrylamide slab gel. Channels are labeled as follows: A = ^{14}C molecular weight standards; B = nonspecific clonal antibody; and C = OKI1 ascites. MW = molecular weight.

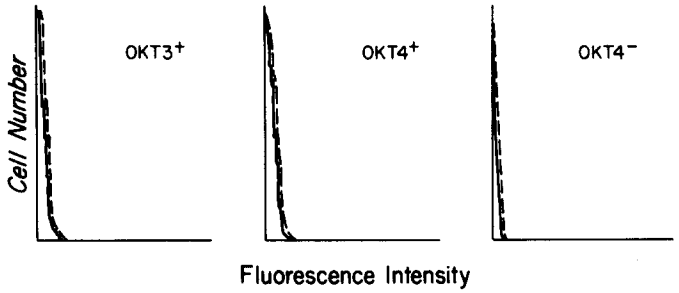

FIG. 3. Absence of Ia-like molecules on resting human T cells. A highly purified T-cell population was obtained by fluorescence-activated cell sorting utilizing a monoclonal hybridoma antibody reactive with the entire human T-cell population (OKT3) or a monoclonal antibody reactive with the human helper cell subset (OKT4$^+$). As shown, neither the OKT3$^+$ T cells, nor the OKT4$^+$ and OKT4$^-$ T cell subsets contained lymphocytes which were reactive by indirect immunofluorescence with OKI1 (solid line). Background fluorescence staining (dotted line) was obtained as in Fig. 1.

significantly smaller percentage of T cells to express Ia than alloantigen. In contrast, tetanus toxoid induced only the OKT4$^+$ T cell subset to express the Ia-like molecule. These results are consistent with the previous observation demonstrating that only the OKT4$^+$ population proliferated to soluble antigen whereas all subpopulations of T cells proliferated to mitogens and alloantigens (12).

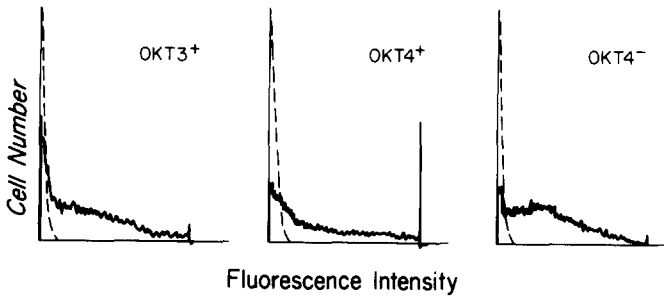

FIG. 4. Expression of Ia-like molecules on allogeneically activated T cells. By indirect immunofluorescence ≥65% of the OKT3$^+$ T cells and OKT4$^+$ or OKT4$^-$ T-cell subsets expressed Ia antigen.

The appearance of Ia-like molecules on T cells may represent either an expansion of a small subset of Ia$^+$ T cells or, in contrast, a de novo biosynthesis of Ia molecules by a significant fraction of the T-cell population. The following experiments were performed in an effort to resolve these possibilities. T cells were obtained and reacted with OKI1 and complement before initiation of MLC with Laz 156. At the end of 5 d, these pretreated, allogeneically stimulated T cells were again analyzed for expression of Ia antigen. As shown in Table II, pretreatment with OKI1 and complement did not effect expression of Ia antigen, and the results were not dissimilar to those obtained from pretreatment with OKI1 alone or control ascites and complement. These results support the view that Ia antigens are not normally expressed on a subpopulation of resting T cells which is then expanded, or that resting T cells have too few Ia determinants to be lysed by OKI1 and complement.

Discussion

This study describes the production and characterization of a monoclonal antibody, termed OKI1, directed at a cell surface molecule found on the vast majority of human B cells and macrophages of all individuals tested. Immunoprecipitation studies revealed that OKI1 reacted with a bimolecular glycoprotein membrane complex of 29,000 and 34,000 dalton subunits. Antigens of a similar biochemical nature and cell surface distribution have been defined by heteroantisera, alloantisera, and monoclonal antibodies to Ia-like molecules in man, guinea pigs, and mice (1–4, 19–27). The reactivity of OKI1 with B lymphocytes from multiple individuals demonstrates a lack of polymorphism in the Ia-like antigen being defined by this monoclonal antibody and suggests that it recognizes a common framework determinant similar to heteroantisera described above (4). In addition to B cells and macrophages, OKI1 reacted with 20–30% of null cells by indirect immunofluorescence. On the other hand, no Ia antigen was detected on resting human T cells isolated by specific monoclonal antibodies defining the entire T-cell population (OKT3$^+$) or the human inducer (helper) subset (OKT4$^+$). However, after mitogen activation with PHA or Con A and alloactivation in MLC, 20 and 70% of T cells, respectively, expressed Ia antigen. Moreover, with these activation stimuli, both the OKT4$^+$ and OKT4$^-$ T-cell subsets expressed Ia antigen to an equivalent degree. In contrast, soluble antigens induced the formation of Ia antigen on 20% of T cells and these T cells were all of the OKT4$^+$ subpopulation.

In prior studies, it was shown that both the OKT4$^+$ and OKT4$^-$ T cell subsets

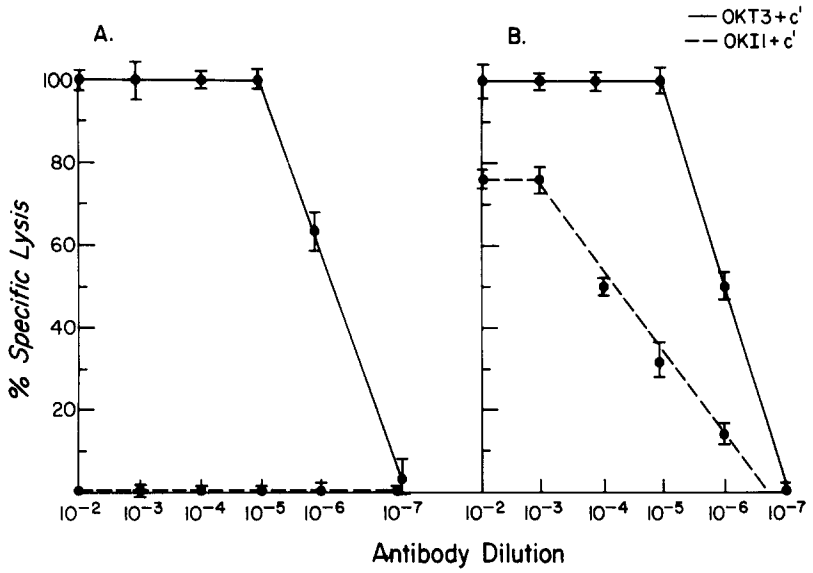

FIG. 5. Lytic capacity of OKI1 and complement on unactivated and activated T cells. As shown, OKT3 and complement are lytic for both unactivated (A) and activated (B) T cells to a dilution of 10^{-6}. In contrast, unactivated T cells are not lysed by OKI1 and complement, whereas activated T cells are lysed to a dilution of 10^{-5} with a maximal lysis of 75% at a dilution of 10^{-3}.

proliferated to optimal mitogenic doses of PHA and Con A and in MLC (12). In contrast, it was shown that only the $OKT4^+$ subset proliferated to soluble antigen. The present finding suggests that only the $OKT4^+$ subset can be induced by tetanus toxoid to express Ia antigen. Thus, the expression of Ia antigen on an individual subset of T cells is clearly linked to the response of that subset to a given activation stimulus. Similarly, it has been shown that individuals with autoimmune or immunodeficiency states who have disorders of inducer or suppressor subsets possess T cells which are Ia activated in vivo (28). Given the fact that the OKI1 monoclonal antibody detected a common Ia antigen framework, it was not possible to determine whether specific subset restricted Ia antigens exist in man. Further characterization and resolution of unique I-region antigens in humans will come from studies with antibodies reactive with restricted regions of the molecule and displayed on subsets of human lymphocytes.

Immunofluorescence studies demonstrated the expression of Ia molecules after activation in MLC and studies utilizing complement mediated lysis supported these findings. Unactivated T cells were not lysed by OKI1 and complement at any dilution tested whereas after activation, T cells were lysed by OKI1 and complement even at an antibody dilution of 1:100,000. Furthermore, the percentage of cells lysed by OKI1 and complement was consistent with that defined by fluorescence. To determine whether the expression of Ia on a large percentage of T cells after activation was the result of clonal expansion of a small subset of T cells expression Ia, T cells were first pretreated with OKI1 and complement. This pretreatment did not eliminate the appearance of Ia^+ cells at the end of 6 d and the results were virtually indistinguishable from studies where the T cells were not pretreated. These findings support the view that clonal expansion is not the major mechanism of Ia expression following activation

TABLE I

Expression of Ia-like Antigens on T Cells after Activation

Activation stimulus	T cells expressing Ia		
	OKT3$^+$	OKT4$^+$	OKT4$^-$
	%	%	%
PHA	18	22	16
Con A	20	16	15
Laz 156_m	70	65	70
Tetanus toxoid	20	38	0

TABLE II

Failure of OKI1 and Complement Pretreatment to Abrogate Expression of Ia-like Antigens on T Cells after Allosensitization

Pretreatment			T cells expressing Ia-like molecules	
OKI1	Control ascites	Complement	MLC stimulated	Media control
			%	%
+	−	+	66	0
+	−	−	68	0
−	+	+	70	0

stimulus. Nevertheless, one could not exclude the possibility that resting T cells have Ia antigens but too few to serve as targets for lysis by anti-Ia and complement.

The present study re-affirms that the Ia molecule in man is not normally expressed on T cells to any appreciable extent. The utilization of T-cell specific monoclonal antibodies to select the T-cell population for analysis provided a highly selective purification procedure. There is the possibility that prior demonstration of Ia on resting T cells could have been due to contaminating macrophages, B cells, or Null cells. While the present study does not exclude the possibility that a small number of T cells bear Ia-like molecules, the percentage of Ia$^+$ T cells would have to be considerably less than 5% of the peripheral T-cell population. Moreover, the synthesis of Ia-like molecules and the de novo expression at the level of the cell surface appears to represent unequivocal evidence of T-cell activation which is linked to the specific activation stimulus and the genetically defined program of the cell responding to that stimulus. The use of a monoclonal antibody directed at an Ia-like framework may provide a useful probe with which to measure immunologic activation of human T lymphocytes.

Summary

The nature of Ia antigens which appear on human T cells after activation and the stimuli required for their expression was examined utilizing a monoclonal antibody reactive with the Ia antigen framework. T cells were purified using monoclonal antibodies directed either at the entire T-cell population (OKT3) or the T-cell inducer subset (OKT4). By indirect immunofluorescence, it was shown that the human T-cell

population contains no detectable Ia^+ cells in the resting state. In contrast, in excess of 60% of the T-cell population expresses Ia antigen after alloactivation in the mixed lymphocyte culture. Moreover, these Ia antigens are expressed within both the $OKT4^+$ and $OKT4^-$ subsets. Similarly, phytohemagglutinin and concanavalin A induced ~20% of peripheral T cells to express Ia antigen and the expression of these antigens is not restricted to either OKT4 subset. In contrast, only the inducer T-cell population which proliferates maximally to soluble antigen expresses Ia antigens after activation by tetanus toxoid. Thus, the expression of human Ia antigens on unique T-cell subsets depends upon the activation stimuli utilized and ability of the individual subset to respond to a given stimulus. Additional studies indicated that Ia antigens appear on previously Ia^- T cells after activation and do not result from clonal expansion of a small subset of Ia^+ T cells.

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References

1. Winchester, R. J., S. M. Fu, P. Wernet, H. G. Kunkel, B. Dupont, and C. Jersild. 1975. Recognition by pregnancy serums of non-HLA alloantigens selectively expressed on B lymphocytes. *J. Exp. Med.* **141:**924.
2. Humphreys, R. E., J. M. McCune, L. Chess, H. D. Herrman, D. J. Malenka, D. L. Mann, P. Parham, S. F. Schlossman, and J. L. Strominger. 1976. Isolation and immunologic characterization of a human B lymphocyte specific cell surface antigen. *J. Exp. Med.* **144:** 98.
3. Winchester, R. J., C. Y. Wang, J. Halper, and T. Hoffman. 1976. Studies with B cell allo- and heteroantisera: parallel reactivity and special properties. *Scand. J. Immunol.* **5:**745.
4. Schlossman, S. F., Chess, L., R. E. Humphreys, and J. L. Strominger. 1976. Distribution of Ia-like molecules on the surface of normal and leukemic cells. *Proc. Natl. Acad. Sci. U.S.A.* **73:**1288.
5. Fu, S. M., N. Chiorazzi, C. Y. Wang, G. Montazeri, H. G. Kunkel, H. S. Ko, and A. B. Gottlieb. 1978. Ia-bearing T lymphocytes in man. Their identification and role in the generation of allogeneic helper activity. *J. Exp. Med.* **148:**1423.
6. Evans, R. L., T. J. Faldetta, R. E. Humphreys, D. M. Pratt, E. J. Yunis, and S. F. Schlossman. 1978. Peripheral human T cells sensitized in mixed leukocyte culture synthesize and express Ia-like antigens. *J. Exp. Med.* **148:**1440.
7. Zimmermann, R., H. Ozer, W. C. DeWolfe, R. L. Evans, E. J. Yunis, and S. F. Schlossman. *Transplant. Proc.* In press.
8. Reinherz, E. L., L. M. Nadler, D. S. Rosenthal, W. C. Moloney, and S. F. Schlossman. 1979. T cell subset characterization of human T-CLL. *Blood.* **53:**1066.
9. Kohler, G., and C. Milstein. 1975. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature (Lond.).* **256:**495.
10. Mendes, N. F., M. C. A. Tolnai, N. P. A. Silveira, R. B. Gilbertson, and R. S. Mitzgar. 1973. Technical aspects of the rosette tests used to detect human complement receptor and sheep erythrocyte binding (T) lymphocytes. *J. Immunol.* **111:**860.
11. Chess, L., R. P. MacDermott, and S. F. Schlossman. 1974. Immunologic functions of isolated human lymphocyte subpopulations. I. Quantitative isolation of human T and B cells and response to mitogens. *J. Immunol.* **113:**1113.

12. Reinherz, E. L., P. C. Kung, G. Goldstein, and S. F. Schlossman. 1979. Separation of functional subsets of human T cells by a monoclonal antibody. *Proc. Natl. Acad. Sci. U.S.A.* **78:**4061.
13. Reinherz, E. L., P. C. Kung, G. Goldstein, and S. F. Schlossman. 1979. A monoclonal antibody with selective reactivity with functionally mature human thymocytes and all peripheral human T cells. *J. Immunol.* **123:**1312.
14. Kung, P. C., G. Goldstein, E. L. Reinherz, and S. F. Schlossman. 1979. Monoclonal antibodies defining distinctive human T cell surface antigens. *Science (Wash. D. C.).* **206:** 347.
15. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage t4. *Nature (Lond.).* **277:**680.
16. Bonner, W. M., and R. A. Laskey. 1974. A film detection method for Tritium-labeled proteins and nucleic acids in polyacrylamide gels. *Eur. J. Biochem.* **46:**83.
17. Julius, M. H., R. G. Sweet, C. G. Fatham, and L. A. Herzenberg. 1974. Mammalian cells: Probes and Problems. Proceedings of the First Los Alamos Life Science Symposium, Atomic Energy Commission Series (CONS 73-1007):107.
18. Giphart, M. J., J. F. Kaufman, A. Fuks, D. Albrechtson, B. G. Solheim, J. W. Brunning, and J. L. Strominger. 1977. HLA-D associated alloantisera react with molecules similar to Ia antigens. *Proc. Natl. Acad. Sci. U.S.A.* **74:**3533.
19. Bodmer, W. F., J. G. Bodmer, J. R. Batchelor, H. Festenstein, and P. G. Morris. 1978. *In* Histocompatibility Testing 1977. Munksgaard, Copenhagen. 22.
20. van Rood, J. J., A. van Leeuwen, J. J. Keuning, and A. Blusse van Oud Alblas. 1975. The serological recognition of the human MLC determinants using a modified cytotoxicity technique. *Tissue Antigens.* **5:**73.
21. Quaranta, V., G. A. Molinaro, C. A. Molinaro, F. Indiveri, R. A. Curry, and S. Ferrone. 1979. Monoclonal DR antibodies. *Fed. Proc.* **38:**1422.
22. Charron, D. J. and H. O. McDevitt. 1979. Production of monoclonal antihuman Ia by somatic cell hybridization and two-dimensional gel electrophoresis (2D-gel) analysis of the HLA-D region products. *Fed. Proc.* **38:**1280.
23. Vitetta, E. S., J. Klein, and J. W. Uhr. 1974. Isolation and partial characterization of Ia antigens from murine lymphoid cells. *Immunogenetics.* **1:**82.
24. David, C. S., C. D. Shreffler, and J. Frelinger. 1973. New lymphocyte antigen system (Lna) controlled by the Ir region of the mouse H-2 complex. *Proc. Natl. Acad. Sci. U.S.A.* **70:**2509.
25. Hammerling, G. J., B. D. Deak, G. Mauve, U. Hammerling, and H. O. McDevitt. 1974. Lymphocyte alloantigens controlled by the I-region of the major histocompatibility complex in mice. *Immunogenetics.* **1:**68.
26. Jones, P. P., D. B. Murphy, and H. O. McDevitt. 1978. Two-gene control of the expression of a murine Ia antigen. *J. Exp. Med.* **148:**925.
27. Schwartz, B. D., W. E. Paul, and E. M. Shevach. 1976. Guinea pig Ia antigens: functional significance and chemical characterization. *Transplant. Rev.* **30:**174.
28. Reinherz, E. L., R. Parkman, J. Rappeport, F. S. Rosen, and S. F. Schlossman. 1979. Aberrations of suppressor T cells in human graft-versus-host disease. *New Engl. J. Med.* **300:** 1061.