POSSIBLE INVOLVEMENT OF THE OKT4 MOLECULE IN T CELL RECOGNITION OF CLASS II HLA ANTIGENS

Evidence from Studies of Cytotoxic T Lymphocytes Specific for SB Antigens

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The relationship between the functional capacity of T cell subsets and the differentiation antigens on their cell surfaces has been the subject of many recent studies in mice and humans. Swain and colleagues (1–3) provided evidence that murine T cells that respond to class I (H-2K and H-2D) major histocompatibility complex (MHC)[1] antigens express high levels of Lyt-2 antigen. This was observed not only for cytotoxic T lymphocytes (CTL) but also for T cells that produce nonspecific helper factors for antibody production as well as T cell growth factor (TCGF) (1–3). Anti-Lyt-2 antibody could block the cytotoxic function as well as the helper cell function and TCGF release of class I-reactive alloimmune T cells (3). Thus, the Lyt-2 molecule is not only expressed on the cell surface but also may play some role in antigen recognition by T cells because it has been observed that anti-Lyt-2 antibodies interfere with the functions of class I-specific T cells (3–10).

In contrast, T cells that respond to class II antigens (H-2I-encoded Ia antigens) were shown to be Lyt-2^-, regardless of their functions, and could not be inhibited with anti-Lyt-2 antibodies (2, 3). These results have led to the conclusion that most, if not all, class I-reactive T cells are Lyt-2^+, and class II-reactive T cells are Lyt-2^-.

Some of these observations in murine systems have been paralleled by results of human studies. Monoclonal antibodies have been developed that detect a differentiation antigen (designated OKT8 or Leu-2) (11–13) that is present on CTL and suppressor-effector cells but is generally absent from cells with helper/inducer functions (14). Like the murine anti-Lyt-2 antibodies, antibodies to the OKT8 molecules have been shown to inhibit allogeneic cytotoxic activity (12, 15, 16) and CTL-target cell conjugate formation (16). We tested and verified the hypothesis that this antibody, like murine anti-Lyt-2, is present on (and interferes with the function of) those CTL that react with human class I antigens but not those CTL that react with human

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[1] *Abbreviations used in this paper:* CTL, cytotoxic T lymphocytes; MHC, major histocompatibility complex; MLC, mixed lymphocyte culture; PBL, peripheral blood lymphocytes; PHA, phytohemagglutinin; TCGF, T cell growth factor.

class II antigens.

Additional monoclonal antibodies define a human T cell differentiation marker (designated OKT4 or Leu 3) (11–13) that is generally found on helper/inducer cells; these cells might be the counterpart of the murine Lyt-2$^-$ cells. The availability of these reagents made it possible to test the hypothesis that the OKT4 antigen on class II-specific CTL is analogous to the OKT8 antigen on class I-specific CTL. Recent findings in several laboratories suggest that OKT4 may mark CTL that are specific for HLA-DR antigens (17, 18), the best characterized human class II gene products. We found evidence to support this hypothesis using CTL directed at SB antigens. These recently discovered HLA antigens are similar to HLA-DR antigens in structure and tissue distribution (19–22); however, the SB gene is distinguishable genetically from HLA-DR (20–22), and its gene product can be separated immunochemically from HLA-DR (22–23). Based on the ability of a series of monoclonal antibodies to the OKT4 molecule to inhibit SB-specific killing, a model is proposed for the role of the OKT4 molecule in interactions between T cells and Ia-positive cells.

Materials and Methods

Human Blood Reagents. Peripheral blood mononuclear leukocytes (PBL) and plasma were obtained by batch leukapheresis of normal adult volunteers and were separated by flotation on Ficoll-Hypaque as described (24). Plasma from 5–10 such male donors was pooled, frozen in aliquots at −20°C, and used as the normal human plasma pool. Fresh PBL were cryopreserved and thawed as previously described (24). HLA-serotyping of cells was kindly performed by Dr. R. J. Duquesnoy, Blood Center of Southeastern Wisconsin, Milwaukee, WI.

Immunochemical Reagents. Purified monoclonal antibodies were prepared from ascites as previously described (11). The monoclonal antibodies used in these studies are OKT1 (IgG1) (25), OKT3 (IgG2a) (11), OKT4 (IgG2b) (11), OKT4A (IgG1) (26), OKT4B (IgM) (26), OKT4C (IgG1) (26), OKT4D (IgG3) (26), OKT8A (IgG2) (27), and OKT9 (IgG1) (28).

Generation and Assay of CTL. Cytotoxic effector cells were generated in one-way mixed lymphocyte culture (MLC) of cells from donors carefully selected to have limited disparity of their HLA histocompatibility antigens. For the SB-specific effectors, the donors were matched for HLA-A, -B, -C, -D, -DR, MB, and MT, but mismatched for SB; donor combinations were identical to those used to generate the standard primed lymphocyte typing reagents that define the SB specificities (19, 29). The details of culture were as previously described (29). For the HLA-A2-specific effectors, matching was for all HLA-A and -B antigens other than HLA-A2. HLA-A2-specific CTL were used after primary or secondary stimulation, whereas the SB-specific CTL were used after secondary or tertiary stimulation. Restimulation in a tertiary response (to amplify cell yield) was performed in a manner analogous to that described for secondary restimulation.

The ^{51}Cr release cytotoxicity assay was performed as previously described (19). Effector cells were assayed at four or more effector-to-target cell ratios. Results are expressed as mean percent specific lysis (19). Standard errors for percent lysis were <5% and are omitted for clarity of presentation. Lytic unit calculations were performed as previously described (23). Target cells were either lymphoblasts prepared by 3-d stimulation of PBL with phytohemagglutinin (PHA M form; Gibco Laboratories, Grand Island Biological Co., Grand Island, NY) or lymphoblastoid B cell lines transformed with Epstein-Barr virus (19). Incubation time for the assays ranged from 3–6 h; in antibody-blocking assays, some effectors were harvested earlier than others in an attempt to achieve unblocked lysis in an optimum range (20–40% specific lysis). Inhibition of cytotoxicity at the effector cell stage was performed by incubation of effectors with monoclonal antibodies for 15–20 min before addition of ^{51}Cr-labeled target cells.

Cell Separations and Immunofluorescence. To eliminate OKT3$^+$, OKT4$^+$, or OKT8$^+$ cells by C′-mediated lysis, cells were incubated in a 1:10^3 dilution of antibody ascites (6×10^6 cells/ml of antibody) for 30 min at 4°C, washed once, resuspended in a 1:10 dilution of normal rabbit serum (6×10^6 cells/ml), incubated at 37°C for 40 min, and washed once. The reactivities of

TABLE I
Specificity of Anti-SB CTL

Responder/ Stimulator‡	E/T§	Percent specific lysis of target cells*									
		E2B	FB5B	FB5T	FB11B	FB11T	H9B	H9T	M4B	M4T	M14B
FB6 anti-FB11	50:1	12	5	2	57	9	35	8	41	14	13
(Anti-SB1)	17:1	6	0	1	55	5	26	8	27	10	5
HLA antigens recognized ‖		—	—	—	SB1	SB1	SB1	SB1	SB1	SB1	—
S11 anti-H9	50:1	3	3	0	43	3	20	4	22	6	1
(Anti-SB1)	17:1	1	1	0	38	3	10	2	15	3	0
HLA antigens recognized		—	—	—	SB1	SB1	SB1	SB1	SB1	SB1	—
W7 anti-H9	50:1	5	2	0	7	1	20	3	26	6	0
(Anti-SB2)	17:1	1	−1	−1	3	1	14	0	15	4	0
HLA antigens recognized		—	—	—	—	—	SB2	SB2	SB2	SB2	—
H9 anti-W7	50:1	8	28	1	3	1	0	0	1	0	15
(Anti-SB4)	17:1	2	13	0	1	0	−1	−1	−1	0	6
HLA antigens recognized		—	SB4	SB4	—	—	—	—	—	—	SB4

* Target cells were either Epstein-Barr virus-transformed B cell lines (B) or PHA-stimulated PBL (T).
‡ Cytotoxic effector cells were generated in tertiary cultures as described in Materials and Methods.
§ Effector/target ratio.
‖ Specificities listed are the only known HLA specificities shared between stimulator and target but absent from responder cells. HLA typing results are as follows: E2: A3, B7, DR1, 4, SB3, MB1, 3; FB5: A25, 33, B15, 17, C3, DR4, 5, SB4, 4; FB6: A25, 33, B15, 40, C3, DR4, 4, SB4; FB11: A25, 31, B15, 40, C3, DR4, 4, SB1; H9: A1, 2, B7, 8, DR2, 3, SB1, 2; M4: A1, 29, B13, 37, C2, 6, DR7, SB1, 2; M14: A1, 2, B7, 8, DR2, 3, SB3, 4; S11: A1, 2, B7, 8, DR2, 3, SB2, 5; and W7: A1, 2, B7, 8, DR2, 3, SB1, 4.

cell populations with monoclonal antibodies (at saturating concentrations) were analyzed by indirect immunofluorescence using a fluorescence-activated cell sorter (FACS IV; B-D FACS Systems, Becton, Dickinson & Co., Sunnyvale, CA), as previously described (30). Cell surface antibody was detected with a fluorescein-conjugated $F(ab')_2$ preparation of goat anti-mouse IgG (heavy and light chain specific, N. L. Cappel Laboratories, Cochranville, PA).

Results

Specificity of CTL. The SB-specific CTL were generated in MLC between donors' cells that were matched for HLA-A, -B, -C, -D, -DR, MB, and MT but were mismatched for SB. The specificity of these responses is well characterized because these combinations of responder and stimulator cells are those originally used to define the SB antigens (19, 29). The specificity of some of these effectors is illustrated in Table I. The effectors lyse only B cells but not T lymphoblasts autologous to the B cells (e.g., 57% vs. 9%, 43% vs. 3%). Lysis is observed on targets that share the sensitizing SB antigen with the stimulator cell but no other defined HLA-A, -B, -DR, MB, or MT antigens. In addition to SB-specific CTL, HLA-A2-specific CTL were generated in MLC by stimulation of donors' cells that are disparate at HLA-A2 but no other HLA-A or -B antigen.

Antibody plus C′ Elimination of CTL. CTL generated against SB1, SB2, SB3, and SB4 were treated with OKT3, OKT4, or OKT8 plus C′ immediately before the

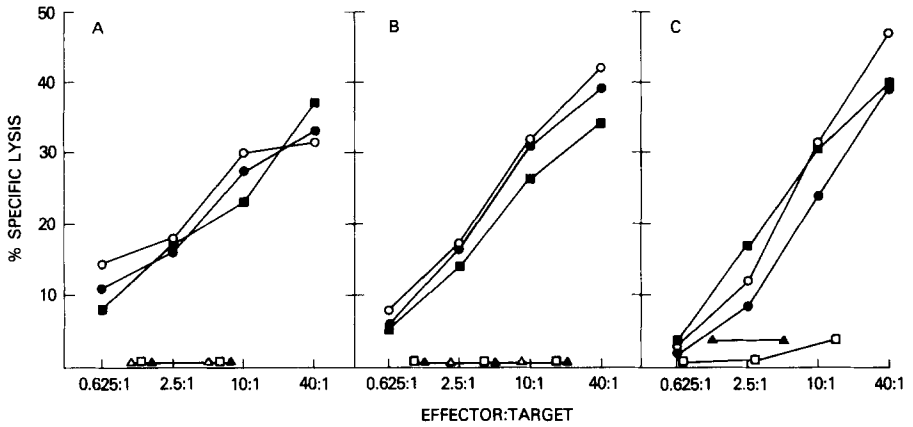

FIG. 1. Treatment of SB-specific CTL with antibody and C′. CTL specific for SB antigens were generated by stimulating responder PBL with irradiated stimulator PBL in culture as follows: panel A, anti-SB2 (W7 anti-H9 assayed on M4 B cell targets); panel B, anti-SB4 (H9 anti-W7 assayed on FB5 B cell targets); and panel C, anti-SB1 (S11 anti-H9 assayed on FB11 B cell targets). HLA typing results for all donors in these panels are given in footnote([11]) to Table I. CTL effector populations were either untreated (●) or treated with C′ alone (○), OKT3 + C′ (△), OKT4 + C′ (□), OKT8 + C′ (■), or OKT4 + OKT8 + C′ (▲), before addition to target cells.

TABLE II

Reactivity of SB-specific and HLA-A2-specific CTL with OKT Antibodies

Antigen recognized	Responder/stimulator*	Target cell	Cell surface phenotype		Relative lytic activity		Percent inhibition by antibody		
			OKT4+	OKT8+	OKT4−	OKT8−‡	OKT8§	OKT3§	OKT4A/4B ‖
			%		%				
SB1	FB6 anti-FB11	H9B	ND¶	ND	<1	>99	14	70	64
	S11 anti-H9	FB11B	ND	ND	<2	>98	9	76	68
SB2	W7 anti-H9	M4B	89	2	<1	>99	6	59	25
	F2 anti-B17	M4B	ND	ND	ND	ND	17	69	13
SB3	W7 anti-M14	M19B	95	7	<3	>97	−3	70	66
SB4	H9 antiW7	FB5B	73	14	<1	>99	24	83	75
	FB11 anti-FB6	W7B	92	7	<3	>99	14	68	45
HLA-A2	Primary FR4/W5	Q1T	61	23	>83	<17	63	85	−27
	Secondary FR4/W5	Q1T	57	43	>86	<14	48	80	9

* SB-specific CTL were generated in secondary and tertiary MLC.

‡ Percent relative lytic activity remaining after treatment with OKT4 plus C′ (OKT4−) or OKT8 plus C′ (OKT8−).

§ Calculated as 100 − (percent specific lysis in the presence of antibody [5 μg/ml]/percent specific lysis in the absence of antibody × 100).

‖ Calculated as percent inhibition by OKT4A (5 μg/ml) + percent inhibition by OKT4B (5 μg/ml) / 2.

¶ Not determined.

addition to appropriate ^{51}Cr-labeled B cell targets. Results of titrating treated and control untreated CTL populations specific for SB1, SB2, and SB4 are illustrated in Fig. 1. All SB-specific CTL activity could be eliminated by treatment with C′ plus OKT3, OKT4, or mixed OKT4 and OKT8, but not by treatment with C′ alone or with OKT8 plus C′. When OKT4− cells were rare in a CTL population, treatment of these populations with OKT4 and C′ resulted in low cell yields, and, consequently, not all of these populations could be tested at the highest effector-to-target ratio (40:1). Comparisons of overall lytic activity (Table II), which take into account both cell yield and cytotoxic activity, indicate that <3% of the recovered activity was contained in the OKT4-treated population and >97% was contained in the OKT8-

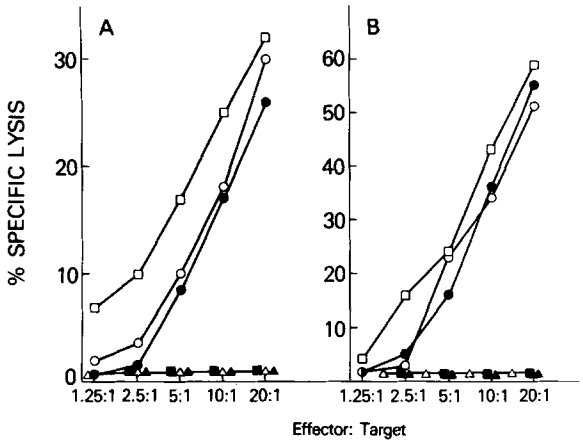

FIG. 2. Treatment of HLA-A2-specific CTL with antibody and C′. Primary (panel A) and secondary (panel B) HLA-A2-specific CTL were prepared by sensitization of FR4 PBL (A1, 1, B8, 8, DR1, 4, SB4) against W5 PBL (A2, B8, DR3, SB2, 4) and assayed on PHA-stimulated targets from donor Q1 (A2, 3, B7, 44, C4, DR1, 7, SB4). CTL effector populations were treated with antibodies and C′, as described in the legend to Fig. 1.

treated population (Table II). FACS analyses before treatment with monoclonal antibodies and C′ indicated that 73–95% of all cells in the SB-specific CTL populations were $OKT4^+$, whereas only 2–14% of these cells were $OKT8^+$ (Table II).

The elimination of SB-specific cytotoxicity by OKT4 but not OKT8 plus C′ contrasted with results previously reported for primary allogeneic CTL responses (14). To assure that these differences were not due to enrichment of or selection for different subpopulations of cells in secondary (and tertiary) responses, HLA-A2-specific CTL were analyzed both in primary and secondary responses (Fig. 2 and Table II). Although the SB-specific cultures contained predominantly $OKT4^+$ cells, the secondary anti-HLA-A2 population contained approximately equal numbers of $OKT4^+$ and $OKT8^+$ cells. Removal of the $OKT8^+$ cells but not the $OKT4^+$ cells eliminated the HLA-A2-specific cytotoxicity generated in both primary and secondary cultures.

Inhibition of Cytotoxicity by Monoclonal Antibodies. Studies of antibody blocking (in the absence of complement) were undertaken to determine whether the OKT8 molecule plays a functional role in both class I-specific and class II-specific cytotoxicity and whether the OKT4 molecule plays a functional role in cytotoxicity of either specificity. SB-specific and HLA-A2-specific cytotoxicity was analyzed in the presence of monoclonal antibody against OKT8 and each of a series of monoclonal antibodies to unique noncompeting epitopes on the OKT4 molecule (OKT4, 4A, 4B, 4C, and 4D).[2] The OKT3 antibody, which is known to inhibit many T cell functions (16, 31–33), was included as a positive control. Two negative controls consisted of OKT1 (25), which reacts with all peripheral T cells, and OKT9 (28), which reacts with the transferrin receptor. Detailed blocking data are shown for six representative effectors (Fig. 3); a simplified summary of these six effectors and others that were tested is provided in Table II. CTL specific for SB1 (Fig. 3, panel A) were strongly inhibited by OKT3 but not by OKT1, OKT8, or OKT9. OKT4A and 4B partially inhibited

[2] Rao, P. E., M. A. Talle, P. Kung, and G. Goldstein. Five epitopes of a differentiation antigen on human inducer T cells distinguished by monoclonal antibodies. Manuscript submitted for publication.

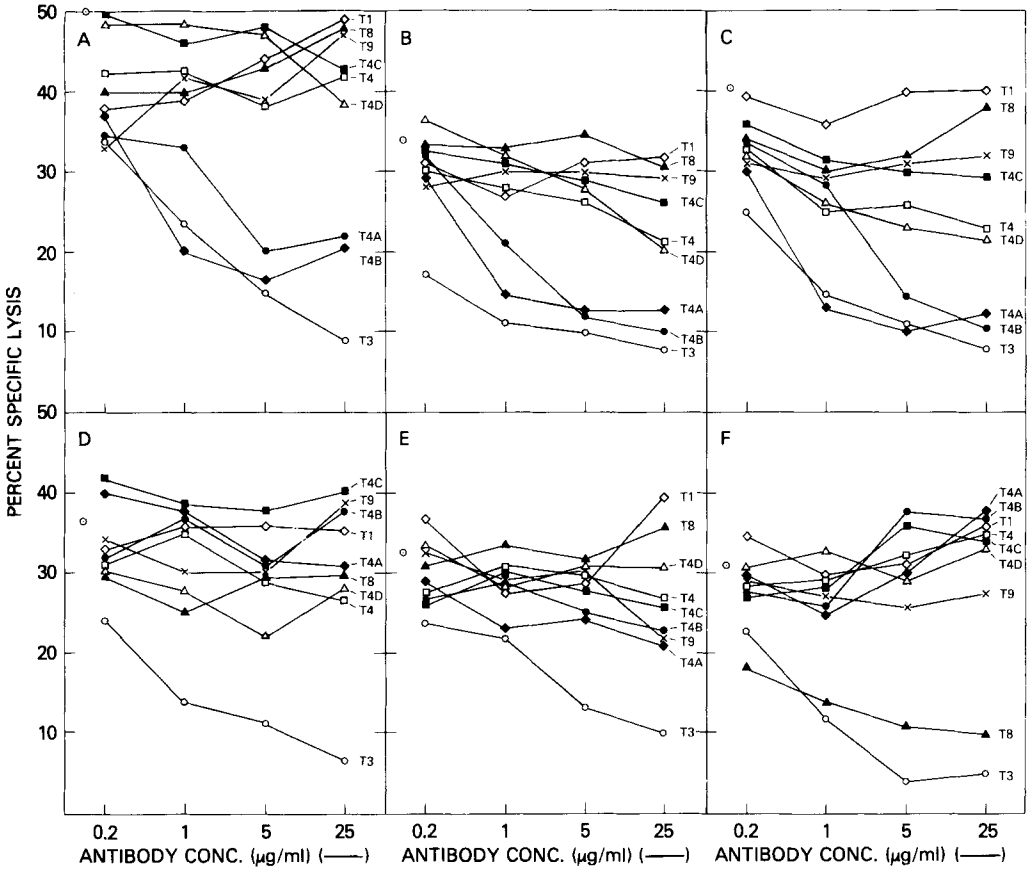

FIG. 3. Inhibition of cytotoxicity by monoclonal antibodies. CTL against SB antigens and HLA-A2 were prepared as follows: anti-SB1 (panel A, FB6 anti-FB11 assayed on H9 B cells); anti-SB3 (panel B, W7 anti-M14 assayed on M19 B cells); anti-SB4 (panel C, H9 anti-W7 assayed on FB5 B cells); anti-SB2 (panel D, F2 anti-UB17 assayed on M4 B cells); anti-SB2 (panel E, W7 anti-H9 assayed on M4 B cells); and anti-HLA-A2 (panel F, FR4 anti-W5 assayed on Q1 T cells). CTL activity was assayed in the absence (⊙) or in the presence of monoclonal antibodies OKT1 (◇), OKT3 (○), OKT4 (□), T4A (◆), T4B (●), T4C (■), T4D (△), OKT8A (▲), and OKT9 (×). All antibody-blocking assays were performed with an effector-to-target ratio of 40:1. HLA typing results for donors not previously given in the legends to Figs. 1 and 2 and Table I are as follows: F2: A1, 2, B7, 8, DR2, 4, SB4, 5; M14: A1, 2, B7, 8, DR2, 3, SB3, 4; M19: A3, 11, B35, 51, C4, SB3, 4; and UB17: A1, 2, B7, 8, DR2, 4, SB2, 4.

SB1-specific cytotoxicity, with a plateau of inhibition observed over a fivefold concentration of antibody between 5 and 25 μg/ml. OKT4, 4C, and 4D were unable to significantly inhibit SB1-specific cytotoxicity. Similar results were obtained for a different SB1-specific CTL population (Table II), an SB3-specific CTL population (Fig. 3, panel B; and Table II), and two different SB4-specific CTL populations (Fig. 3, panel C; and Table II).

In contrast, two different anti-SB2-specific CTL populations (Fig. 3, panels D and E; and Table II) were strongly inhibited by OKT3 but were not well blocked by any of the OKT4 antibodies. The inability of OKT4A and 4B to block anti-SB2-specific killing is not due to an absence of OKT4A and 4B on these cells because >95% of the

cells in the CTL population in panel E bound OKT4, 4A, and 4B antibodies by FACS analysis (data not shown). The lack of inhibition with OKT4A and 4B is apparently a function of the specificity of the CTL rather than of the genetics of the responder cell because the same responder cells (W7) that were sensitized against SB3 (W7 anti-M14, Fig. 3, panel B; and Table II) and against SB2 (W7 anti-H9; Fig. 3, panel E; and Table II) were differentially inhibited by OKT4A and 4B.

HLA-A2-specific CTL (Fig. 3, panel F; and Table II) were not inhibited by any of the OKT4 antibodies, but were inhibited by OKT3 and OKT8.

Discussion

The results of the present studies demonstrate that in six SB-specific CTL populations tested, many more cells express OKT4 antigens than OKT8 antigens, and >95% of the SB-specific cytotoxic activity is mediated by cells that are eliminated by OKT4 plus complement but not by OKT8 plus complement. These data indicate that OKT4 may be expressed on SB-specific but not on HLA-A2-specific CTL. Although it is possible that the cells that mediate SB-specific killing express low levels of OKT8 and are therefore resistant to treatment with OKT8 and complement, flow microfluorometric analysis of cells after treatment with antibody and complement has provided no evidence for this hypothesis (data not shown). Because some CTL express detectable levels of OKT8 (but not OKT4) and other CTL express detectable levels of OKT4 (but not OKT8), expression of these differentiation antigens does not correlate with function but may correlate with specificity. This possibility is strengthened by the combined results of these studies and those of Krensky et al. (17), which indicate that CTL lines specific for DRw6 were $OKT4^+8^-$, but control populations specific for HLA-A2 or -B7 were $OKT4^-8^+$. Thus, CTL specific for products of two distinct Ia-like genes, SB and DR, express OKT4 but not OKT8. Conversely, CTL specific for class I antigens express OKT8 but not OKT4. It would appear that the OKT4 marker may be expressed on most T cells that recognize allogeneic Ia antigens or self Ia plus foreign antigens; these $OKT4^+$ cells do not appear to be functionally homogeneous in that they can act both as helper/inducer and cytotoxic cells.

Data from at least two laboratories suggest that OKT4 may be expressed on CTL clones that apparently are not specific for class II antigens. Moretta et al. (34) have demonstrated that CTL clones derived from MLC can have the phenotypes $OKT4^-8^-$, $OKT4^+8^-$, or $OKT4^-8^+$. The specificity of these effectors was not defined, but because all of the $OKT4^+8^-$ CTL clones produced >37% lysis of PHA-induced lymphoblasts, they presumably were not specific for class II antigens. Pawalec et al. (35) have also observed $OKT4^+8^-$ clones that strongly lysed peripheral blood mononuclear cells. It is uncertain whether the expression of T cell differentiation antigens on long-term cultured cells maintained with TCGF accurately reflects normal differentiation or whether unusual events predominate, such as selection for rare cell types or expression of aberrant phenotypes. Thus, the OKT4 marker may be present on most if not all class II-specific CTL as well as some long-term CTL clones with no apparent class II specificity.

Studies of CTL inhibition by monoclonal antibodies suggest that the OKT4 molecule is not only expressed on SB-specific CTL, but it may be involved in CTL-target cell interactions (recognition and lethal hit). The OKT4A and 4B antibodies were able to inhibit the lysis of five SB-specific CTL populations by 45–75%. These

results indicate that the OKT4A and 4B antibodies interfere with effector T cell function because these antibodies cannot bind to the B cell targets. The OKT4 molecule apparently is not an essential component of the lytic machinery because it is not present on HLA-A2-specific CTL. The simplest hypothesis is that the OKT4 molecule is involved in antigen recognition by SB-specific CTL because its presence and function correlate with antigen specificity (i.e., class I vs. class II). This hypothesis is supported by two findings: (*a*) antibodies against a molecule (Leu-3) apparently identical to OKT4 inhibited proliferation of Leu-3^+ cells in MLC (36); and (*b*) preliminary results indicate that OKT4A and 4B can block SB-specific proliferative responses (unpublished observations). Interference with antigen recognition could be the mechanism by which antibody is able to inhibit both T cell proliferation and cytotoxicity.

Three elements of complexity need to be considered in the data on antibody-mediated inhibition. First, blocking by OKT4A and 4B is not complete but reaches a plateau of inhibition over a fivefold range of antibody concentrations. These results could reflect the fact that the antibodies are not binding directly to the T cell antigen recognition unit, but rather that they bind to epitopes near the recognition unit and cause partial steric inhibition. Alternatively, there may be clonal heterogeneity of SB-specific CTL, such that only a portion of the CTL clones are susceptible to inhibition. A precedent for such clonal heterogeneity has been provided by MacDonald et al. in their studies of inhibition of CTL clones by anti-Lyt-2 antibodies (10). The second complexity is that OKT4, 4C, and 4D bind to the same molecule as OKT4A and 4B,[2] but are unable to inhibit CTL activity. These results could indicate that the OKT4, 4C, and 4D antibodies have a lower affinity than 4A and 4B for the OKT4 molecule or that the OKT4, 4C, and 4D epitopes are located at some greater distance from the antigen-recognition site on the CTL. The third complexity, lack of inhibition of SB2-specific cytotoxicity by OKT4A and 4B antibodies, is open to several different explanations. The most provocative hypothesis to explain this apparent antigen specificity of inhibition is that the OKT4 antibodies bind to a site on the T cell that is in close proximity to the binding site of the T cell antigen-specific receptors that recognize some but not other Ia antigens. However, preliminary results of antibody inhibition of proliferation indicate that SB2-specific proliferation, like other SB-specific proliferation, is partially inhibitable by OKT4A and 4B (unpublished observations). These results indicate that there may not be anything fundamentally unique about SB2-specific T cell recognition. Instead, postulates such as higher affinity of SB2-specific effectors or unique geometry of SB2-specific CTL recognition are ad hoc explanations for the failure to block SB2-specific CTL.

Despite the foregoing complexities, the results presented in this report are consistent with the basic concept that the OKT4 molecule may be involved in T cell recognition of class II HLA antigens. Data from other studies also suggest that the OKT4 molecule is functionally involved in T cell-Ia antigen interactions. The findings that OKT4^+8^- cells are located adjacent to Ia^+ cells in lymphohematopoietic organs and gut mucosa (37, 38) indicates that $OKT4^+$ cells possess a cell surface mechanism that enables them to preferentially associate with Ia^+ cells. Perhaps the OKT4 molecule provides a low affinity recognition mechanism by which these cells home to and survey the limited population of Ia^+ cells in the body. Such a homing or focusing mechanism would facilitate reactivity of these cells with Ia^+ antigen-presenting cells.

As a stimulus for discussion, we propose two possible molecular models for the interaction of $OKT4^+$ T cells with Ia antigens (Fig. 4): one in which the OKT4 molecule is the T cell receptor for class II antigens and another in which it acts in concert with the T cell receptor. In both models, we propose that an invariant structure on the OKT4 molecule interacts with an invariant structure on the Ia molecule. Such an interaction of nonpolymorphic structures, which is characteristic of most receptor-ligand interactions outside the immune system, would be efficient from an evolutionary and genetic point of view because it would assure satisfactory interaction of class II-specific T cells with autologous Ia^+ cells in individuals of any genotype. It is possible that the antigen-specific T cell receptor and this invariant Ia receptor are on the same polypeptide chain (the OKT4 molecule) (Fig. 4, panel A). Alternatively, the OKT4 molecule and the T cell receptor could be distinct cell surface structures but would be intimately associated during Ia antigen recognition (Fig. 4, panel B). Structural studies of the heterogeneity of OKT4 will be required to differentiate between these two possibilities.

Summary

A recently described HLA gene, SB, which maps between GLO and HLA-DR, codes for Ia-like molecules that are similar to but distinct from HLA-DR molecules. Cytotoxic T lymphocytes (CTL) specific for SB1, SB2, SB3, and SB4 were compared with HLA-A2-specific CTL with respect to their surface expression of the T cell differentiation antigens OKT3, OKT4, and OKT8. All CTL activity was eliminated by treatment with OKT3 and C′. The SB-specific cytotoxicity was eliminated by OKT4 plus C′ but not by OKT8 plus C′. In contrast, HLA-A2-specific killing was completely susceptible to treatment with OKT8 plus C′ but not with OKT4 plus C′. Cytotoxicity was analyzed in the presence of OKT8 and a series of monoclonal antibodies (OKT4A, 4B, 4C, and 4D) that react with distinct epitopes on the OKT4 molecule. SB1-, SB3-, and SB4-specific CTL were partially inhibited by OKT4A and 4B (45–75%), whereas HLA-A2-specific CTL were partially inhibited by OKT8 (48–63%) but not by OKT4. SB2-specific CTL were not inhibited (<26%) by OKT8 or by any of the OKT4-related antibodies. These results suggest that the OKT4 marker may be expressed on most T cells that recognize allogeneic Ia or self Ia plus foreign antigens; $OKT4^+$ cells do not appear to be functionally homogeneous in that they

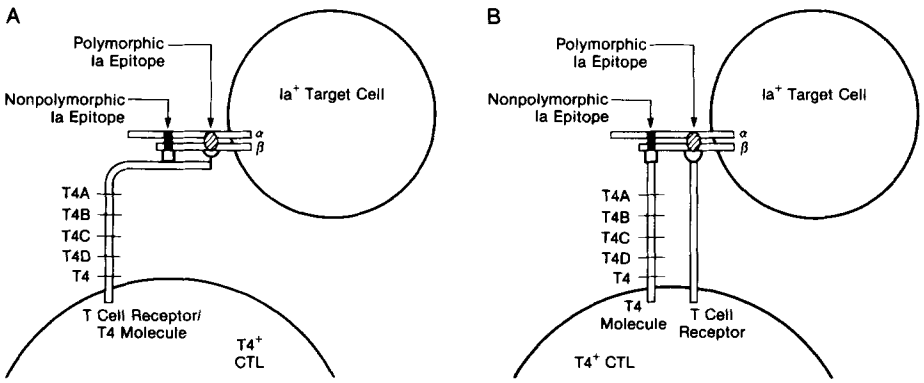

FIG. 4. Models for the functional involvement of OKT4 molecules in Ia recognition by T cells.

can act both as helper/inducer and cytotoxic cells. Models are proposed for the functional involvement of the OKT4 molecule in T cell-Ia antigen interactions.

Note added in proof: Meuer et al. (*Proc. Natl. Acad. Sci. U. S. A.* **79:**4395) and Ball and Stastny (*Immunogenetics*, in press) have recently described class II-specific OKT4$^+$CTL.

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