Brief Definitive Report

ANTIGEN-LIKE EFFECTS OF MONOCLONAL ANTIBODIES DIRECTED AT RECEPTORS ON HUMAN T CELL CLONES*

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Using antigen-specific human T lymphocytes as immunogens, it has been possible to generate monoclonal antibodies against clonally unique surface determinants expressed on a novel class of 90-kdalton Ti molecules. Each surface Ti molecule exists as a heterodimer comprised of a covalently linked 49-kdalton α- and 43-kdalton β-chain and, in addition, is membrane-associated with the 20/25-kdalton T3 molecule (1–3). Perhaps more importantly, in contrast to the monomorphic T3 structure, differences in proteolytic cleavage and isoelectric-focusing analyses of Ti structures from clones of unrelated specificities suggest that peptide variability exists within the latter (2).

Given the fact that monoclonal antibodies to the Ti structure of clones of unrelated specificities are non-cross-reactive and by themselves inhibit antigen-specific clonal function, it is likely that anti-Ti monoclonal antibodies define variable regions of the T cell antigen receptor (3). If such a notion were correct, then it might be anticipated that under the appropriate conditions, anti-Ti antibodies could induce clonal T cell activation in a fashion analogous to antigen. In the present study, this possibility was investigated by triggering T cell clones with purified anticlonotypic monoclonal antibodies coupled to the surface of a solid support (Sepharose 4B) and this mode of activation compared with that induced by cell surface bound antigen itself. In the results to be reported, we demonstrate that analogous to antigen, anticlonotypic monoclonal antibodies selectively induce clonal proliferation and lymphokine production.

Materials and Methods

Generation and Culture of Human T Cell Clones. The human alloreactive T cell clones $CT4_{II}$ and $CT8_{III}$ were derived by stimulating lymphocytes of a healthy donor with the allogeneic Epstein-Barr virus (EBV)-transformed lymphoblastoid B cell line, Laz 156. Cloning and recloning were performed as previously described (4).

Generation of Monoclonal Antibodies. The following monoclonal antibodies were utilized in the present study: anti-$T3_B$ (all mature T lymphocytes and a fraction of thymocytes); anti-$T4_A$ and anti-$T4_B$ (human inducer population); anti-$T8_A$ and anti-$T8_B$ (human suppressor population); anti-T11 (E rosette positive human lymphocytes). Anti-Ti_{1B} represents a clone-specific antibody uniquely recognizing $CT8_{III}$, and anti-Ti_{2A} is clonotypic for $CT4_{II}$ cells. Both monoclonals have been shown to be non-cross-reactive, however, recognizing

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analogous T3-associated 90-kdalton (49/43) heterodimers on their corresponding T cell clones (1–3). Moreover, they inhibit antigen-specific clonal function and enhance the clone's responsiveness to interleukin 2 (IL-2). All monoclonal antibodies were used in ascites form at final dilutions of 1:250 to 1:2,500 in culture medium.

Purification and Surface Coupling of Monoclonal Antibodies. Monoclonal antibodies of IgG isotypes (anti-$T3_B$, anti-$T4_A$, anti-$T4_B$, anti-$T8_A$ and anti-$T8_B$) were purified employing Sepharose protein A (Pharmacia, Uppsala, Sweden) according to the method of Ey (5). Anti-Ti_{1B} and anti-Ti_{2A} (both IgM) were purified from ascites on Sephadex G-200 columns (Pharmacia). Following purification, the various monoclonal antibodies were individually coupled to CnBr-activated Sepharose 4B (Pharmacia) at a concentration of 3 mg of purified antibody per ml of swollen Sepharose beads. The amount of antibody coupled per bead was similar for all reagents produced. The functional integrity of each surface-linked monoclonal antibody was tested by its ability to immunoprecipitate the appropriate antigen from ^{125}I surface-labeled T cell clones.

Proliferative Assays. 3×10^4 $CT4_{II}$ or $CT8_{III}$ cells were incubated in triplicate cultures with medium, IL-2, 3×10^4 Laz 156 cells or 3×10^4 Laz 475 cells (both 5,000 rad irradiated) in round-bottomed microtiter wells (Costar, Cambridge, MA). To determine proliferative effects of Sepharose-linked monoclonal antibodies, beads were suspended at 1:4 dilutions (bead/volume) in phosphate-buffered saline, washed three times, and then further diluted in culture medium. Proliferative responses to these reagents were dose dependent and could be observed with <10 beads/well. Following 24 h of in vitro incubation at 37 C, 7% CO_2, humidified atmosphere, cultures were individually pulsed for 16 h with 1 μCi of tritiated thymidine per well, harvested on a MASH II apparatus (M. A. Bioproducts, Walkersville, MD) and ^3H-TdR incorporation was measured in a Packard liquid scintillation spectrometer (Packard Instrument Co., Inc., Downer's Grove, IL).

Determination of IL-2 Secretion by $CT4_{II}$ and $CT8_{III}$. $CT4_{II}$ or $CT8_{III}$ at 0.5×10^6 per ml were individually incubated with medium or an identical number of irradiated Laz 156 cells (5,000 rad irradiated), or monoclonal antibodies coupled to Sepharose beads at concentrations that were found to induce optimal clonal proliferation. After 24 h, supernatants were harvested, passed through 0.22-μm filters and IL-2 activity calculated according to the method of Gillis (6), using an unrelated IL-2-dependent human T cell clone. This activity was compared with a purified IL-2 standard kindly provided by Dr. Kendall Smith (Dartmouth Medical School).

Results and Discussion

To determine whether anticlonotypic monoclonal antibodies could specifically activate clonal T cell function, we used two alloreactive cytotoxic T cell clones, $CT4_{II}$ and $CT8_{III}$, and a series of non-cross-reactive anticlonotypic monoclonal antibodies to each termed, respectively, anti-Ti_{2A-C} and anti-Ti_{1A-B}, which are directed at their unique T3-associated 90-kdalton heterodimers (1–3). The two alloreactive clones were obtained from a single donor following stimulation of peripheral blood mononuclear cells with the EBV-transformed B lymphoblastoid line Laz 156. Each clone has been maintained in long-term culture for approximately 2 years without changing phenotype, function, or specificity (1, 4). $CT4_{II}$ has been previously shown to be T3+T4+T8− and recognizes a class II MHC gene product on the surface of Laz 156 cells. In contrast, $CT8_{III}$ is T3+T4−T8+ and directed at a class I alloantigen on Laz 156 (1, 4). Both clones, in addition to mediating specific cytotoxicity, proliferate to the allogeneic cell line Laz 156.

Because the alloantigens that serve as receptor ligands are membrane bound and likely interact via multi-point surface attachment and because previous studies indicated that anticlonotypic monoclonal antibodies, by themselves, were not mitogenic for $CT4_{II}$ and $CT8_{III}$, we investigated the functional effects of

purified monoclonal antibodies bound to a solid surface support. To this end, anti-$T3_B$, anti-$T4_A$, anti-$T4_B$, anti-$T8_A$, anti-$T8_B$, anti-Ti_{2A}, and anti-Ti_{1B} were purified and covalently linked to CnBr-activated Sepharose beads. Subsequently, Sepharose-bound antibodies were incubated with one or another T cell clone and proliferative responses to these beads, Laz 156, and IL-2-containing media measured in parallel (Fig. 1). As expected from previous studies, $CT4_{II}$ and $CT8_{III}$ proliferated both to irradiated Laz 156 cells as well as to IL-2-containing media, but not to the irrelevant B-lymphoblastoid target Laz 475. More importantly, Sepharose anti-Ti_{2A} and anti-Ti_{1B} stimulated a selective proliferation of $CT4_{II}$ and $CT8_{III}$, respectively, which is comparable to that obtained with the allogeneic cell Laz 156. In contrast, neither anti-T4 nor anti-T8 Sepharose-bound antibodies had any mitogenic effect. In addition, Sepharose anti-T3 induced a proliferative response in both clones.

Since in previous studies it was found that unbound anti-Ti and anti-T3 antibodies could inhibit the proliferative response of reactive clones to antigen, we next examined the effect of free monoclonal antibodies in solution on the response of $CT8_{III}$ to surface-bound anti-Ti_1 or anti-T3. As shown in Fig. 2*A*, addition of the $CT8_{III}$-specific anticlonotype anti-Ti_{1B} or anti-T3 completely blocks proliferation of $CT8_{III}$ to Sepharose anti-Ti_{1B}. In contrast, anti-T8 and the unreactive anticlonotypic anti-Ti_2 have no inhibitory effects. Moreover, as shown in Fig. 2*B*, free anti-T3 monoclonal antibody or anti-Ti_B inhibit proliferation of $CT8_{III}$ to Sepharose-bound anti-T3 antibodies. In an analogous fashion, anti-T3 or the $CT4_{II}$-specific anticlonotype anti-Ti_2 abrogates the response of

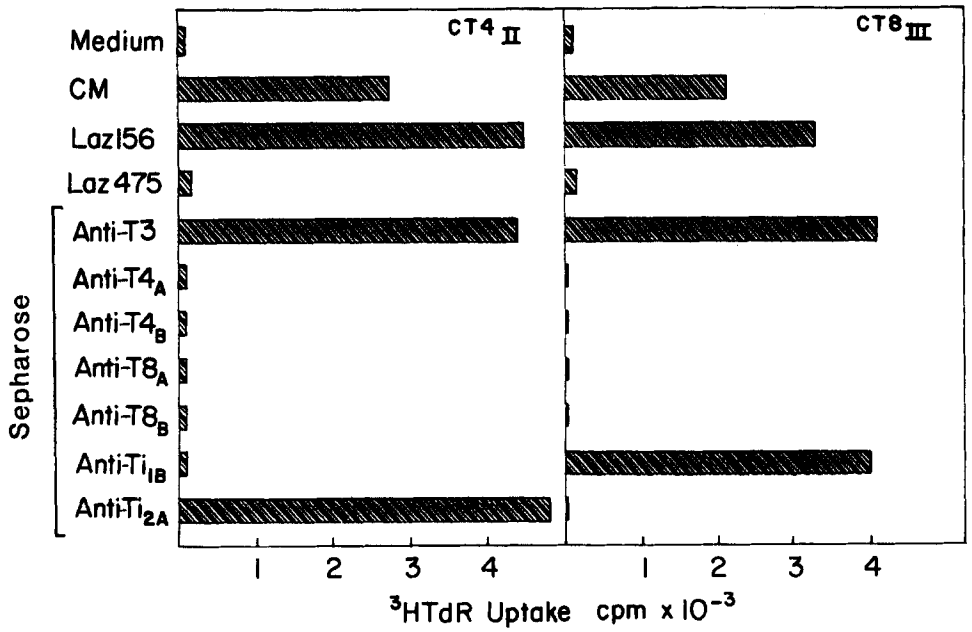

FIGURE 1. Induction of clonal proliferation by surface-bound monoclonal antibodies. $CT4_{II}$ or $CT8_{III}$ cells (3×10^4/well) were incubated with medium, Laz 156 cells, Laz 475 cells (3×10^4/well; 5,000 rad irradiated), IL-2-containing supernatants (CM) (5% final concentration) or monoclonal antibodies covalently coupled to Sepharose-4B beads. Cultures were individually pulsed after 24 h with 1 μCi of ^{3}H-TdR and harvested 16 h later. Values represent means of triplicates. Standard deviations were $\leq$12%.

$CT4_{II}$ cells to Sepharose-bound anti-Ti_2, whereas anti-$T4_A$ and anti-Ti_{1B} do not (data not shown). These results provide further support for the notion that the monomorphic T3 structure and the clonotypic structure are intimately linked in the T cell membrane and together serve a central role in antigen-specific T cell activation. The critical importance of T3 observed at the clonal level is consistent with earlier findings at the heterogeneous population level (7–11).

From the above results it is clear that $CT8_{III}$ and $CT4_{II}$ can be induced to proliferate to specific antigen, Sepharose-linked anti-T3 or Sepharose coupled with the relevant anti-Ti antibodies in the absence of exogenous lymphokines. To next determine whether such activation might induce endogenous lymphokine production, as recently demonstrated using antiidiotypic heteroantisera directed at murine T cell clones (12), $CT8_{III}$ or $CT4_{II}$ were incubated for 24 h at 37 C with either irradiated Laz 156 or Sepharose-linked anti-Ti_{1B}, anti-Ti_{2A}, and anti-T3, respectively. Subsequently, supernatants were harvested and IL-2 activity calculated in units per milliliter according to the method of Gillis et al. (6). As shown in Table I, alloantigen stimulation induced the release of significant amounts of IL-2 from both $CT4_{II}$ and $CT8_{III}$. More importantly, Sepharose-bound anti-T3 and Sepharose-bound anti-Ti directed at the relevant specificity (i.e. anti-Ti_1 for $CT8_{III}$ and anti-Ti_2 for $CT4_{II}$), also induced IL-2 secretion. It should be noted that the same culture supernatants could induce proliferation of $CT8_{III}$ and $CT4_{II}$ as well (data not shown). Control supernatants from clones and stimulator combinations that did not result in clonal proliferation (i.e. Sepharose-anti-T4 and Sepharose-anti-T8) (Fig. 1) contained no detectable IL-2 activity. The finding that the T8+ clone $CT8_{III}$ and the T4+ clone $CT4_{II}$ each produce lymphokine upon specific stimulation confirms earlier findings that both human T cell subsets contain populations capable of secreting IL-2 (14).

A number of important points emerge from these experiments: (*a*) Antigen

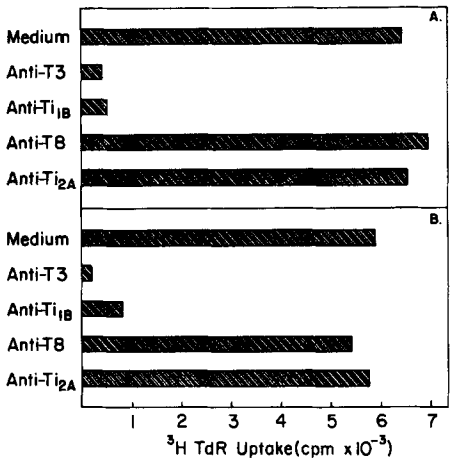

FIGURE 2. Inhibition of $CT8_{III}$ proliferation to Sepharose anti-T3 and Sepharose anti-Ti_{1B} by monoclonal antibodies in solution. 3×10^4 $CT8_{III}$ cells were incubated for 30 min at room temperature with uncoupled monoclonal antibodies or medium before addition of optimal amounts of Sepharose anti-Ti_{1B} (*A*) or anti-T3 (*B*). Final concentrations of free monoclonal antibody ascites were 1:500 in all cases. Following 24 h at 37 C, cultures were individually pulsed with 1 μCi of ^{3}H-TdR and harvested 16 h later. Values represent means of triplicates. Standard deviations were $\leq$12%.

TABLE I

Lymphokine Secretion by $CT4_{II}$ and $CT8_{III}$

Stimulus	T cell clone	
	$CT4_{II}$	$CT8_{III}$
Laz 156	1.3*	1.2
Sepharose anti-T3	1.6	1.5
Sepharose anti-Ti_{1B}	<0.1	1.8
Sepharose anti-Ti_{2A}	2.0	<0.1

* IL-2 (U/ml).

Triggering of lymphokine secretion from $CT4_{II}$ and $CT8_{III}$. Following incubation of $CT4_{II}$ or $CT8_{III}$ cells (5×10^6/ml) with one or another stimulus for 24 h supernatants were harvested, passed through 0.22-μm filters, and subsequently analyzed for IL-2 activity using an IL-2-dependent human T cell clone. IL-2 activity was quantitated according to Gillis et al. (6). Purified human IL-2 was used as a reference.

(Laz 156), anti-Ti and anti-T3 monoclonal antibodies produce very similar functional effects with regard to clonal proliferation and lymphokine secretion; (*b*) Triggering of a single clonally unique epitope, Ti_1 on the surface of $CT8_{III}$ or Ti_2 on $CT4_{II}$, appears to be sufficient to induce antigen-specific functions; (*c*) Multimeric interaction between ligand and antigen receptor is an essential requirement for the initiation of clonal T cell responses because non–surface-linked monoclonal antibodies do not mediate these effects; (*d*) The inhibitory effects of free anti-T3 monoclonal antibodies on the response to Sepharose-anti-Ti beads further stresses the importance of the 20/25-kdalton surface molecule in the process of T cell activation and its linkage to the clonotypic T cell surface structure; (*e*) The T4 and T8 surface structures, although critical for MHC-restricted cytotoxic T lymphocyte (CTL) effector function (4, 8, 13), are not likely involved in induction of clonal proliferation or lymphokine secretion; and (*f*) Clonal proliferation to antigen or anticlonotypic antibody is due, at least in part, to endogenous lymphokine secretion. Moreover, given the observation that these clones respond to IL-2-containing media as well, it is clear that a single cell can, under physiologic conditions, both produce and respond to its own lymphokine(s).

Taken together, these results provide compelling evidence to support the notion that anti-Ti antibodies define the antigen receptor structure on individual clones. Binding studies with isolated Ti material will now be necessary to further substantiate this view.

Summary

Recent studies suggested that the clonally unique Ti epitopes defined by non-cross-reactive monoclonal antibodies might represent the variable regions of the antigen receptor. Here we determine whether such anti-Ti antibodies could trigger clonal T cell activation. Anticlonotypic monoclonal antibodies to the 49/43-kdalton heterodimer of a given clone or antibodies to the 20/25-kdalton membrane associated monomorphic T3 molecule selectively induce proliferation and IL-2 secretion when linked to a solid support. In contrast, anti-T4 and anti-T8 antibodies under the same conditions have no effect. In conclusion, these results imply that anticlonotypic antibody functions in a fashion analogous to antigen and further support the notion that the T3-Ti molecular complex represents the antigen receptor on human T lymphocytes.

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References

1. Meuer, S. C., K. A. Fitzgerald, R. E. Hussey, J. C. Hodgdon, S. F. Schlossman, and E. L. Reinherz. 1983. Clonotypic structures involved in antigen-specific human T cell function: relationship to the T3 molecular complex. *J. Exp. Med.* 157:705.
2. Reinherz, E. L., S. C. Meuer, K. A. Fitzgerald, R. E. Hussey, J. C. Hodgdon, O. Acuto and S. F. Schlossman. 1983. Comparison of T3-associated 49/43KD cell surface molecules on individual human T cell clones: evidence for peptide variability in T cell receptor structures. *Proc. Natl. Acad. Sci. USA.* 80:4104.
3. Meuer, S. C., O. Acuto, R. E. Hussey, J. C. Hodgdon, K. A. Fitzgerald, S. F. Schlossman, and E. L. Reinherz. 1983. Evidence for the T3-associated 90KD heterodimer as the T cell antigen receptor. *Nature (Lond.)* 303:808.
4. Meuer, S. C., S. F. Schlossman, and E. L. Reinherz. 1982. Clonal analysis of human cytotoxic T lymphocytes: T4+ and T8+ effector T cells recognize products of different major histocompatibility complex regions. *Proc. Natl. Acad. Sci. USA.* 79:4395.
5. Ey, P. L., S. J. Prowse, and C. R. Jenkin. 1978. Isolation of pure IgG1, IgG2a and IgG2b immunogobulins from mouse serum using protein A-Sepharose. *Immunochemistry.* 15:429.
6. Gillis, S., M. M. Ferm, W. Ou, and K. A. Smith. 1978. T cell growth factor: parameters of production and a quantitative microassay for activity. *J. Immunol.* 120:2027.
7. Reinherz, E. L., S. Meuer, K. A. Fitzgerald, R. E. Hussey, H. Levine, and S. F. Schlossman. 1982. Antigen recognition by human T lymphocytes is linked to surface expression of the T3 molecular complex. *Cell.* 30:735.
8. Meuer, S. C., R. E. Hussey, J. C. Hodgdon, T. Hercend, S. F. Schlossman, and E. L. Reinherz. 1982. Surface structures involved in target recognition by human cytotoxic T lymphocytes. *Science (Wash. DC).* 218:471.
9. Reinherz, E. L., R. E. Hussey, and S. F. Schlossman. 1980. A monoclonal antibody blocking human T cell function. *Eur. J. Immunol.* 10:758.
10. van Wauwe, F. P., J. R. DeMay, and J. G. Goossener. 1980. OKT3: a monclonal anti-human T lymphocyte antibody with potent mitogenic properties. *J. Immunol.* 124:2708.
11. Burns, G. F., A. W. Boyd, and P. C. L. Beverley. 1982. Two monoclonal anti-human T lymphocyte antibodies have similar biologic effects and recognize the same cell surface antigen. *J. Immunol.* 129:1451.
12. Infante, A. J., P. D. Infante, S. Gillis, and C. G. Fathman, 1982. Definition of T cell idiotypes using anti-idiotypic antisera produced by immunization with T cell clones. *J. Exp. Med.* 155:1100.
13. Reinherz, E. L., R. E. Hussey, K. A. Fitzgerald, P. Snow, C. Terhorst, and S. F. Schlossman. 1981. Antibody directed at a surface structure inhibits cytolytic but no suppressor function of human T lymphocytes. *Nature (Lond.)* 294:168.
14. Meuer, S. C., R. E. Hussey, A. C. Penta, K. A. Fitzgerald, B. M. Stadler, S. F. Schlossman, and E. L. Reinherz. 1982. Cellular origin of interleukin 2 (IL-2) in man: evidence for stimulus-restricted IL-2 production by T4+ and T8+ T lymphocytes. *J. Immunol.* 129:1076.