1974, compared to the warmer, more saline waters of the period 1948 to 1963 (*16*), represents in the multiyear mean just such a change in circulation. Similar trends were observed over the same period in the Labrador Sea (*4*). Even though such changes may be subject to short-term reversal (*4, 16*), their long-term effect shows clearly in the salinity of deep waters away from the formation regions, which in a sense are smoothed or averaged during spreading of the new dense components from their sources. In the surface samples from the three northernmost TTO legs, salinities were lower than 1955–1965 values almost everywhere along the track. In the Labrador Sea the 1981 surface salinities were more than 1 per mil below the 1962 values. [Examination of seasonal trends in surface salinity at ocean weather ships (*3, 4*) reveals that as much as half of the observed difference may result from seasonal variations in surface salinity.] It is also noteworthy that the TTO cruise track north of Iceland was changed due to an unexpectedly large ice extent.

In summary, between 1962 and 1981 there was a significant climatic freshening in North Atlantic Deep Waters north of 50°N, and an even larger freshening of shallower waters. We suggest that investigators examine ties of the low-salinity Arctic outflows to long-term atmospheric cooling, or examine shifts in the patterns of circulation that might increase the transport of this low-salinity water away from the boundaries and into the regions of water mass formation. The significant hydrographic response to modest short-term climatic forcing points to the importance of quantifying and understanding the nature of water mass formation in these regions.

P. G. BREWER
Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543
W. S. BROECKER
Lamont-Doherty Geological Observatory, Palisades, New York 10964
W. J. JENKINS, P. B. RHINES
Woods Hole Oceanographic Institution
C. G. ROOTH
Rosenstiel School of Marine and Atmospheric Science, Miami, Florida 33149
J. H. SWIFT
Scripps Institution of Oceanography, La Jolla, California 92093
T. TAKAHASHI
Lamont-Doherty Geological Observatory
R. T. WILLIAMS
Scripps Institution of Oceanography

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Identification of the Receptor for Antigen and Major Histocompatibility Complex on Human Inducer T Lymphocytes

Abstract. *Human T cell clones and monoclonal antibodies directed at their surface structures were used to define the receptor for the antigen and major histocompatibility complex on inducer T lymphocytes. The results indicated that the receptor is a single complex consisting of the monomorphic T3 molecule with a molecular weight of 20,000 to 25,000 and a clonotypic disulfide linked heterodimer Ti with a molecular weight of 90,000. Sepharose-bound monoclonal antibodies (anti-Ti_4 or anti-T3) to the receptor could activate clonal proliferation and inducer function for B cell immunoglobulin secretion and thus substitute for the appropriate combination of major histocompatibility complex gene product and specific antigen.*

The initiation of immune responses is critically dependent on the presence of a mature subpopulation of peripheral T lymphocytes termed "inducer T cells." These cells express their individual regulatory programs only upon activation by a specific set of signals consisting of foreign- or autoantigen and autologous class II gene product of the major histocompatibility complex (MHC) (*1*). Since interactions among T cells, antigen, and MHC gene products occur in a highly selective fashion, it has been postulated that each inducer T lymphocyte must possess one or more clonally unique surface recognition receptors. However, there is considerable controversy on whether the T cell receptor reacts with antigen in association with MHC (altered self theory or single receptor model) or antigen and MHC independently (dual recognition theory or two receptor model).

Characterization of inducer T cell receptors would be valuable in understanding the molecular basis for the pivotal roles which these cells play in T-T, T-B, and T-macrophage interactions within the lymphoid system (*2*) as well as their inductive effects on hematopoietic stem cells, fibroblasts, osteoclasts, and other cell types extrinsic to the lymphoid system (*3*). Such information might also provide insights into inducer cell defects in acquired and congenital immunodeficiency states on the one hand or inducer cell hyperactivity in autoimmune disease and atopic disorders on the other (*2*).

To delineate surface recognition structures for antigen or MHC gene products, or both, on inducer T lymphocytes, we generated a series of human inducer T cell clones (phenotype T3+T4+T8−) and subsequently used one of them, termed RW17C, as an immunogen for the generation of monoclonal antibodies. The RW17C clone has been propagated in vitro by stimulation with a combination of ragweed antigen E (RWAGE), autologous antigen-presenting cells (APC's), and interleukin 2 (IL-2) for more than 8 months. As shown in Table 1, RW17C proliferates in response to RWAGE only in the context of autologous APC's as judged by [^{3}H]thymidine incorporation. In contrast, incubation with either autologous or allogeneic APC's alone, or a combination of RWAGE plus allogeneic APC's or autologous APC's plus the unrelated antigen, tetanus toxoid (TT), does not lead to proliferation of RW17C. The same MHC and antigen combination is required to induce helper function for B cells from clone RW17C. Thus, only the combination of RW17C, autologous APC's, and RWAGE results in B cell immunoglobulin G (IgG) production.

RW17C has clear MHC restriction for an autologous class II molecule since antibody to Ia (I-region associated) determinants but not to HLA determinants inhibits clonal proliferation (*4*). Given that RW17C is derived from the T4+ subset and previous results indicating that T4+ cells generally react with antigen in the context of class II molecules, these findings are not unexpected (*5*, *6*).

After immunization of mice with RW17C, a series of murine monoclonal antibodies was produced against the RW17C clone. Hybridoma cultures containing antibody, reactive with RW17C but unreactive with an autologous EBV-transformed B lymphoblastoid line (Laz 509), were selected, cloned, and recloned by limiting dilution in the presence of feeder cells. Malignant ascites were then developed and used for analysis. One individual monoclonal antibody, termed anti-Ti_{4A}, was obtained (one of approximately 500 hybridomas) that did not react with resting or activated T cells or a series of additional T cell clones derived from the same individual ($N = 25$). Nevertheless, the surface structure defined by anti-Ti_{4A} comodulated with the T3 molecule of molecular weights 20,000 to 25,000 (20K to 25K) following incubation of RW17C cells with either anti-Ti_{4A} or any of several anti-T3 monoclonal antibodies tested, including one, termed anti-$T3_C$, generated in the above fusion.

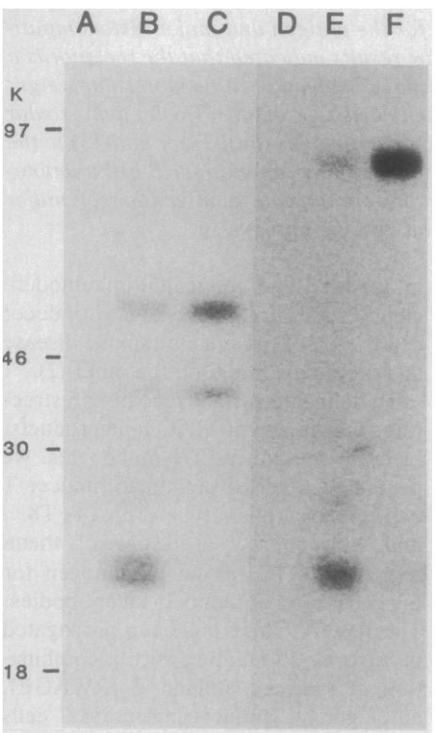

Fig. 1. Characterization of Ti_{4A} and $T3_C$ surface structures. RW17C cells (30×10^6) were surface labeled with 1 mCi of $Na^{125}I$ (New England Nuclear) and immunoprecipitated as previously described (*8*) with the monoclonal antibodies anti-T6, anti-Ti_{4A}, and anti-$T3_C$ covalently linked to CnBr-activated Sepharose 4B. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) was performed in reducing and nonreducing conditions in a 10 percent polyacrylamide gel according to a modification of the Laemmli procedure as described (*8*). The following ^{14}C-methylated molecular weight markers (New England Nuclear) were used: phosphorylase B (97,400); ovalbumin (46,000); carbonic anhydrase (30,000); lactoglobulin A (18,300). Lanes A to C show reducing conditions: A, anti-T6; B, anti-$T3_C$; C, anti-Ti_{4A}; D to F show nonreducing conditions: D, anti-T6; E, anti-$T3_C$; F, anti-Ti_{4A}.

To characterize the surface structure recognized by anti-Ti_{4A} on RW17C, we performed immunoprecipitation experiments with membrane preparations externally labeled with ^{125}I. As shown in Fig. 1, anti-Ti_{4A} precipitates two bands under reducing conditions in SDS-PAGE, one at 52K and a second at 41K (lane C), and appears as a single band at about 90K under nonreducing conditions (lane F). These molecular weight species are not present in control immunoprecipitates with anti-T6 (lanes A and D) or anti-Ti_{4A} from ^{125}I-labeled clones of differing specificities than RW17C, for example, T15A (not shown). In contrast to anti-Ti_{4A}, anti-$T3_C$ precipitates four bands on SDS-PAGE under reducing conditions from RW17C (lane B). The major protein band has a molecular weight of 20K with three additional bands at 25K, 41K, and 52K. Moreover, the two bands of higher molecular weight are identical in size to those found in anti-Ti_{4A} immunoprecipitates from RW17C (lane C), suggesting that they may be related. Further support for this notion comes from the observation that, like Ti_{4A} under nonreducing conditions, the 52K and 41K upper bands detected by anti-$T3_C$ appear as a band at about 90K (lane E). Note that the 20K and 25K species within the anti-$T3_C$ precipitate are similar under both reduced and nonreduced conditions.

These findings are analogous to those previously obtained with a series of human cytotoxic T cell clones. There, it was shown that the 20K to 25K T3 molecule was involved in both the antigen specific proliferation and the cytotoxic lymphocyte (CTL) effector function of all T cells (*7*). In addition, each T3 molecule was itself membrane associated with one clonally unique 90K disulfide-linked heterodimer, termed Ti, consisting of a 49K to 51K α subunit and a 43K β subunit (*8*). Although Ti molecules from T4+ and T8+ clones of differing specificities were comparable in molecular characteristics, they expressed clonally unique epitopes as detected by non-cross-reactive monoclonal antibodies. Furthermore, Ti could be detected in SDS-PAGE of anti-T3 immunoprecipitates from ^{125}I-labeled CTL as evidenced by sequential precipitation studies (*9*). In contrast, anti-Ti_{4A} (lanes C and F), as well as the other anti-Ti antibodies (*9*), does not precipitate detectable material in the range of the 20K to 25K T3 molecule, implying that binding of the monoclonal antibody anti-Ti_{4A} may dissociate the clonotypic structure from the 20K to 25K molecules. Given the above T3 association and the subunit composition of Ti_{4A}, it appears that the clonotype on an MHC-restricted antigen-specific inducer clone is very similar in molecular characteristics to the clonotypes found on CTL clones (*8*). It is noteworthy, however, that the exact molecular weights of the Ti α and β chains of RW17C (Ti_4; 52K and 41K) differ slightly from those found on clone $CT4_{II}$ (Ti_2; 51K and 43K) or clone $CT8_{III}$ (Ti_1; 49K and 43K) (*8*). Comparable structures have now been defined in murine systems as well (*10*).

The clonotypic nature of the Ti_{4A} structure suggested that it might define a unique recognition molecule on the RW17C clone. Given the fact that RW17C reacts with antigen exclusively in the context of an autologous MHC

Table 1. Antigen/MHC restriction of clone RW17C. The human T cell clone RW17C is one of a series of 16 clones derived from a donor with ragweed allergy. Peripheral blood mononuclear cells (PBMC) were incubated with RWAGE (Worthington Diagnostic) and cloned in a semisolid agar system (*5*). Individual colonies were expanded in liquid culture by restimulation with RWAGE, 5000 rad of irradiated autologous PBMC, and IL-2 containing supernatants for more than 8 months (*5*). Further cloning was performed by limiting dilution technique at one cell per well. The surface phenotype of RW17C was T1+T3+T4+T8−T11+T12+Ia+ as analyzed by indirect immunofluorescence on an Epics V cell sorter (*8*). In addition to the RWAGE specific clones, seven T cell clones specific for tetanus toxoid (TT), including T15A, were generated from the same donor.

Stimulus	[^{3}H]Thymidine uptake (count/min)	IgG secretion (ng/ml)
Autologous APC's		
Medium	243	336
RWAGE	5593	7864
TT	90	452
Allogeneic APC's		
Medium	200	415
RWAGE	281	500
TT	221	326

gene product, the Ti_{4A} clonotype could have been the receptor for antigen, the receptor for MHC structures, or the receptor for both. To address this point, we examined the ability of anti-Ti_{4A} and anti-$T3_C$ to trigger clonal proliferation and inducer function. Since antigen is recognized by T cells on the surface of APC's, probably through multimeric attachment, purified monoclonal antibodies covalently linked to the surface of Sepharose-4B beads were used as stimuli. As shown in Table 2, Sepharose-linked anti-Ti_{4A} stimulates in vitro proliferation of RW17C in the absence of APC's or RWAGE. In addition, Sepharose-bound anti-$T3_C$ induces proliferative responses in RW17C. However, whereas the anti-Ti_{4A} reagent has a selective effect on RW17C, Sepharose-linked anti-$T3_C$ activates any T cell clone tested (see legend to Table 2). In contrast, Sepharose-bound anti-T4 and the unrelated anti-Ti_{2B} have no mitogenic effects on RW17C.

Unlike the immobilized monoclonal antibodies, soluble anti-Ti_{4A} completely and selectively inhibits antigen/MHC–induced proliferation of RW17C. Furthermore, as expected, anti-$T3_C$ blocked all antigen specific clones tested [see (*2*) and (*4*)].

The observation that Sepharose anti-$T3_C$ induces clonal activation regardless of the antigen specificity of the responder T cell is not surprising since the 20K to 25K structure appears to represent a constant subunit of the T cell receptor (*7, 8*). In contrast, the unique ability of anti-Ti_{4A} Sepharose to activate RW17C is in keeping with the clonotypic nature of Ti_{4A}. These findings imply that the T3-Ti structure contains the receptor for MHC plus antigen on RW17C.

To determine whether triggering of the T3-Ti_{4A} surface structures on RW17C by monoclonal antibodies could replace both antigen and MHC in the induction of helper function for B cell IgG production as well, RW17C was added to autologous B cells with one or another Sepharose-conjugated monoclonal antibody. As shown in Table 2, both Sepharose-coupled anti-T3 and anti-Ti_{4A}, but not Separose-coupled anti-Ti_{2B}, trigger RW17C helper activity. As with clonal proliferation, anti-Ti_{4A} selectively activates RW17C since a tetanus-specific inducer clone T15A derived from the same donor as RW17C is not induced to provide help with Sepharose-coupled anti-Ti_{4A} (not shown). Sepharose-coupled anti-T4 failed to trigger proliferation or help from either T4+ clone even though the 62K T4 glycoprotein is probably involved as an associative recognition structure for class II molecules and is present on inducer T cells (*2, 5, 6*). That the T4 molecule indeed facilitates an important interaction between RW17C and APC is, however, suggested by the finding that the presence of anti-T4 in soluble form inhibits clonal activation [see (*4*)].

Table 2. Induction of proliferation and regulatory function of RW17C by surface-coupled monoclonal antibodies. Monoclonal antibodies were produced after immunization of BALB/cJ mice with RW17C cells and somatic fusion of immune splenocytes with the murine NS-1 myeloma cell line as described elsewhere (*8*). The individual monoclonal antibodies anti-Ti_{4A}, anti-$T3_C$, anti-Ti_{2B} (*8*), and anti-$T4_A$ (all IgG1) were purified from malignant ascites with protein A-Sepharose (Pharmacia). Five milligrams of purified antibody was coupled to 1 ml of swollen CnBr-activated Sepharose 4B beads (Pharmacia) in each case. Proliferative responses of 3×10^4 T15A or 3×10^4 $CT4_{II}$ were: (i) T15A: + medium, 486 count/min; + Sepharose-linked anti-Ti_{4A}, 367 count/min; + Sepharose-linked anti-$T3_C$, 6012 count/min; + Sepharose-linked anti-Ti_{2B}, 458 count/min. (ii) $CT4_{II}$: + medium, 214 count/min; + Sepharose-linked anti-Ti_{4A}, 116 count/min; + Sepharose-linked anti-$T3_C$, 12,112 count/min; + Sepharose-linked anti-Ti_{2B}, 10,694 count/min. Induction of IgG secretion was determined with 5×10^4 autologous or allogeneic B lymphocytes [E rosette–negative PBMC treated with anti-T4, anti-T8, anti-Mo1, and rabbit complement (*13*)]. To these cells, 2.5×10^4 cloned cells and optimal concentrations of surface-bound monoclonal antibodies were added. Total IgG was determined and culture conditions were as described in Table 1. Control incubation of autologous B lymphocytes, RW17C, and RWAGE (10 μg/ml) resulted in 2509 ng of secreted IgG.

Stimulus	[^{3}H]Thymidine uptake (count/min)	IgG secretion (ng/ml)	
		Autologous B	Allogeneic B
Medium	593	85	96
Sepharose-linked anti-Ti_{4A}	6985	1638	2669
Sepharose-linked anti-$T3_C$	7291	4863	4738
Sepharose-linked anti-Ti_{2B}	552	51	96
Sepharose-linked anti-$T4_A$	635	33	30

The observation that Sepharose-bound anti-T3 and anti-Ti induce both proliferation and helper function suggests that the receptor for antigen and MHC is linked to an as yet undefined regulatory element. Whether this molecule is cytoplasmic or surface bound on RW17C is not known. Nevertheless, the association is intriguing in view of recent results indicating that Sepharose-coupled antibodies to clonotypic determinants, specific for alloreactive clones, are capable of inducing clonal proliferation and IL-2 secretion (*11*). Taken together, these findings suggest that a variety of T cell activities are dependent on triggering of the antigen receptor structure. Furthermore, the inability of antibodies to the receptor (either anti-T3 or anti-Ti_{4A}) in soluble form to activate clonal function suggests that multimeric attachment and receptor cross-linking is critical to this activation process.

As noted above, activation of RW17C by RWAGE requires a specific MHC gene product expressed on autologous APC's. To address the question of whether MHC restriction applies only to the inductive phase of the immune response or also exists at the effector stage of T and B cell interaction, RW17C was triggered with Sepharose-linked anti-Ti_{4A} or anti-$T3_C$ and helper function for autologous versus allogeneic B cells measured by quantitation of IgG output. As shown in Table 2, the triggering of the RW17C T cell receptor with anti-$T3_C$ or anti-Ti_{4A} induces helper function for autologous as well as allogeneic B cells. Moreover, Sepharose-coupled anti-$T3_C$ triggers several other antigen-specific inducer clones ($N = 4$) to provide help for allogeneic B cells (data not shown). The present findings, therefore, support the view that MHC restricted, antigen-specific T lymphocytes interact in a genetically unrestricted fashion at the level of the B cell (*12*). Whether some other clones manifest genetic restriction at the effector stage is not known.

In addition to providing information about the MHC restrictions of regulatory clones, the present clonal populations and antibodies against receptors should assist in the characterization of putative antigen-specific and isotype-specific helper function. Moreover, it should be possible to define the precise relation of such specific helper factors to the surface Ti molecule. Further understanding of the function of allergen (for example, RWAGE) reactive clones at the molecular level should provide insights into the atopic state.

STEFAN C. MEUER
DAVID A. COOPER
JAMES C. HODGDON
REBECCA E. HUSSEY
KATHLEEN A. FITZGERALD
STUART F. SCHLOSSMAN
ELLIS L. REINHERZ
Division of Tumor Immunology, Dana-Farber Cancer Institute, and *Department of Medicine, Harvard Medical School, Boston, Massachusetts 02115*

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Bromine Residue at Hydrophilic Region Influences Biological Activity of Aplysiatoxin, a Tumor Promoter

Abstract. *Aplysiatoxin and debromoaplysiatoxin, which are isolated from the seaweed,* Lyngbya gracilis, *differ in their chemical structure only by the presence or absence of a bromine residue in the hydrophilic region. The function and the structure-activity relation of the hydrophilic region are not known. Aplysiatoxin increased malignant transformation, stimulated DNA synthesis, and inhibited the binding of phorbol-12,13-dibutyrate and epidermal growth factor to cell receptors. Debromoaplysiatoxin inhibited the binding of these two substances as strongly as aplysiatoxin but did not increase malignant transformation or stimulate DNA synthesis. These results indicate that a slight change in the chemical structure of the hydrophilic region of aplysiatoxin affects its abilities to increase cell transformation and stimulate DNA synthesis and that the abilities of the tumor promoters to inhibit the binding of phorbol-12,13-dibutyrate and epidermal growth factor are dissociable from their abilities to increase cell transformation and stimulate DNA synthesis under some circumstances.*

The presence of many kinds of carcinogens, initiators, and tumor promoters in the human environment has been widely recognized. Sugimura *et al.* (*1*, *2*) screened environmental substances to seek new tumor promoters, first by testing the irritancy of the substance on mouse ear, and second by testing the substance for induction of ornithine decarboxylase (ODC) activity in mouse skin. Some compounds that showed high activity in these two tests did not promote tumors when the compounds were painted on mouse skin. The most interesting of these compounds is a polyacetate derivative, debromoaplysiatoxin, the chemical structure of which differs from that of aplysiatoxin only by a lack of bromine residue at the hydrophilic region. Both aplysiatoxin and debromoaplysiatoxin were isolated from a seaweed, *Lyngbya gracilis*. Debromoaplysiatoxin did not improve the adhesion of human promyelocytic leukemia cells (HL-60) to the substrate, whereas aplysiatoxin showed high activity in all of the tests described above, including the test of promoting skin tumors in vivo.

The sequential process and the mechanisms by which diverse effects are induced by the skin tumor–promoting agents are not well understood. The difference between the effects of aplysiatoxin and debromoaplysiatoxin may shed light on the sequential process of promoter action and the functional role of the hydrophilic region of promoters. However, the effects of promoters vary markedly depending on the type and kind of cells and the genetic and physiological condition of the cells (*3*). It is difficult to link diverse observations obtained with different systems or cells. Exclusive use of transformable cultured

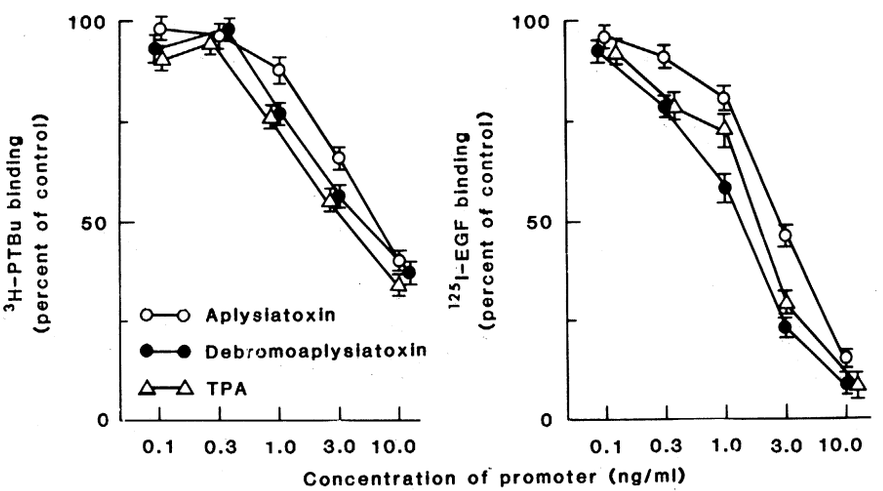

Fig. 1. Inhibition of PDBu and EGF binding by tumor promoter. Cells (2×10^6 per dish) were incubated with 2 ml of Eagle's minimum essential medium (MEM) containing ovalbumin (2 mg/ml), various concentrations of tumor promoter, and 0.2 ng of ^{125}I-labeled EGF (120 mCi/mg; Collaborative Research) for 45 minutes or 5.8 ng of ^{3}H-labeled PDBu (35 mCi/mg; New England Nuclear) for 30 minutes. The cells were then washed three times with MEM containing ovalbumin (2 mg/ml) and lysed with 0.1*N* NaOH, and cell-bound radioactivity was determined.