overall immunocompetence (*19*). Children who experienced protein malnutrition as early infants show deficient serum IgM production even after years of rehabilitation (*20*). The timing of the nutritional deficits—that is, whether malnutrition commenced prenatally or postnatally—plays a significant role in determining the severity and reversibility of the resulting immunodeficiency.

Our most notable observation, then, was the persistence of immunodeficiency in F_1, F_2, and F_3 offspring despite restitution with a diet adequate in all nutrients. Caloric restriction has been reported to have a similar impact on the immunocompetence of F_1 and F_2 progeny (*11*). A similar phenomenon was observed when dietary protein restriction altered brain DNA content and behavior and learning performance in F_2 progeny (*21*). The mechanism whereby zinc or other nutrients influence immune ontogeny in subsequent generations remains obscure. Germ cells obtained from zinc-deprived animals might be studied in vitro to identify the cause of these developmental defects.

This study has important implications for public health and human welfare, as the consequences of fetal impoverishment may persist despite generations of nutritional supplementation. Dietary supplementation beyond the levels considered adequate might allow for more rapid or complete restoration of immunocompetence.

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Surface Structures Involved in Target Recognition by Human Cytotoxic T Lymphocytes

Abstract. *Cloned human cytotoxic T lymphocytes and monoclonal antibodies inhibiting their function (anti-$T3_A$, anti-$T4_A$, and anti-$T8_A$) were used to elucidate the role of T cell surface glycoproteins in cell-mediated lympholysis involving individual classes of gene products of the major histocompatibility complex on target cells. The results indicate that several surface molecules are required for specific target recognition: T3 and T4 on T4+ cytotoxic T lymphocytes and T3 and T8 on T8+ cytotoxic T lymphocytes.*

Cell-mediated lympholysis (CML) is a process whereby T lymphocytes specifically destroy target cells to which they have been sensitized. This mechanism appears to be of importance in allograft rejection, tumor destruction, and lysis of syngeneic cells infected by virus. However, despite recent advances in understanding physiologic requirements and cellular processes of this effector function, the molecular mechnisms involved in target cell recognition and lysis are ill defined (*1*).

In man, cytotoxic effector cells are derived from either of the two major T cell subpopulations. These have been termed T4+ or T8+ on the basis of their uniquely expressed 62,000-dalton (62KD) (T4) and 76KD (T8) glycoproteins (*2–4*). More important, the target antigens recognized by individual subsets are the product of different gene regions of the major histocompatability complex (MHC). Thus, allosensitized T4+ T cells kill target cells bearing class II MHC antigens whereas T8+ T cells kill targets expressing class I MHC antigens (*4*). Similar observations regarding differences in cytotoxic T lymphocyte (CTL) specificity of individual T cell subsets have been observed in the murine system with the homologous Lyt2− and Lyt2+ populations (*5*).

This association between the surface phenotype (that is, surface glycoproteins) of CTL and the class of MHC molecules recognized implies that subset-restricted structures may be required to facilitate selective lysis of different target antigens. This view has been supported by recent findings showing that monoclonal antibodies to the T4 or T8 glycoproteins selectively inhibit cytotoxic effector function of T4+ or T8+ CTL clones (*4*). In addition, a 20KD T cell surface molecule, T3, present on all mature T lymphocytes, participates in cell-mediated lympholysis: antibodies to this structure block killing by both T4+ and T8+ CTL clones.

Whether such surface molecules serve as recognition elements, or alternatively, represent components of the lytic mechanism is unknown. To address this question, we utilized cloned populations of T4+ and T8+ CTL and examined the ability of monoclonal antibodies to the surface structures (T3, T4, and T8) to influence killing under various experimental conditions. Because appropriate concentrations of lectin can induce approximation of CTL and target cells in the absence of antigen recognition (*6*), it is possible to assess the intrinsic killing capacity of CTL clones even in the presence of monoclonal antibodies that inhibit cytolytic function. We reasoned that if the antibodies bind to a surface structure related to the lytic mechanism itself, then artificial approximation should not be capable of reconstituting effective lysis. However, if these anti-

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bodies block structures required for target recognition or appropriate killer-target binding, lectin approximation should result in effective target lysis.

As shown in Table 1, cells from the T4+ clone $CT4_{II}$ and the T8+ clone $CT8_{III}$ killed the human B lymphoblastoid line Laz 156 to which they had been sensitized. Thus, at an effector-target ratio of 20:1, killing by $CT4_{II}$ and $CT8_{III}$ was 47 and 68 percent, respectively. This cytotoxic activity was specific because neither clone mediated the lysis of unrelated targets, for example, Laz 509. After incubation of $CT4_{II}$ and $CT8_{III}$ with monoclonal antibody to $T3_A$ (anti-$T3_A$), killing was reduced by approximately 75 percent (12 to 14 percent specific lysis) in both cases. In contrast, the monoclonal antibodies to $T4_A$ (anti-$T4_A$) and $T8_A$ (anti-$T8_A$) selectively inhibited the killing of that clone which expressed the T4 or T8 determinants. No blocking effects were observed with other monoclonal antibodies including anti-T12 (Table 1) and anti-T1 (not shown).

Perhaps more important, the inhibitory effects of all three monoclonal antibodies were reversed by culturing CTL and target cells with lectin. In the presence of concanavalin A (Con A), the lytic activity of $CT4_{II}$ cells treated with anti-$T3_A$ or anti-$T4_A$ increased from 12 and 9 percent, respectively, to 38 and 36 percent. Restoration of lytic activity from 14 and 6 percent, respectively, to 49 and 46 percent, was also observed after lectin approximation of the target cells and the $CT8_{III}$ clone preincubated with anti-$T3_A$ or anti-$T8_A$. That the $HT4_I$ helper T cell clone did not become cytotoxic (Table 1) suggests that killing is a consequence of the cell's functional repertoire rather than the capacity of Con A to bridge two cell surfaces. This observation further stresses the heterogeneity of cells within the human T4+ T cell subset with respect to the existence of cytotoxic and noncytotoxic populations.

These results imply that at least several surface molecules are important in CML: T3 and T4 molecules on T4+ and T3 and T8 molecules on T8+ clones. Since CML is restored by lectin even in the presence of monoclonal antibodies to these molecules, it appears that T3, T4, and T8 are involved in recognition events rather than the lytic mechanism. In further support of this notion is the observation that the target cell specificity of CTL clones is abrogated by lectin approximation: in the presence of Con A, $CT4_{II}$ and $CT8_{III}$ kill unrelated target cells (Laz 509, Table 1). For example, with lectin, $CT4_{II}$ lyses the irrelevant target Laz 509 (37 to 41 percent specific lysis). Similar results are seen with $CT8_{III}$ (47 to 50 percent). Moreover, under these conditions the killing of both the irrelevant target Laz 509 and the specific target Laz 156 are comparable.

One might argue that the inhibitory effects of these monoclonal antibodies on CTL function are indirect and occur as a consequence of antibody-induced agglutination of cells or steric blockade of still undefined but functionally important surface determinants. The former possibility is not likely since additional antibodies to T cell surface structures, including anti-T1 and anti-T12, do not inhibit CML. Moreover, none of the antibodies blocked other in vitro functions of $CT4_{II}$ and $CT8_{III}$ such as proliferation in response to lymphokines (data not shown). In addition, incubation of T

Table 1. Target recognition by individual human cytotoxic T cell clones. $CT4_{II}$, $CT8_{III}$, and $HT4_I$ represent human alloreactive T cell clones generated from a single healthy donor's lymphocytes stimulated in mixed lymphocyte culture by the EBV-transformed human lymphoblastoid B cell line Laz 156 as described (*4*). The clones were grown in liquid culture for more than 1 year with stable phenotype and function and were recloned at low density. (Phenotype of $CT4_{II}$ and $HT4_I$: T1 + T3 + T4 + T8 − T10 + T11 + T12 + Ia +; phenotype of $CT8_{III}$: T1 − T3 + T4 − T8 + T10 + T11 + T12 + Ia +.) Cytotoxicity of $CT4_{II}$ is directed at class II alloantigens whereas $CT8_{III}$ specifically kills class I alloantigens as confirmed by blocking studies with antiserums to class I and class II antigens (*4*). None of these clones exhibits natural killer or antibody-dependent cellular cytotoxicity activity. Laz 156 (HLA type A2, A3; B7, B40; Dr2, Dr4) and Laz 509 (HLA type A2, A25; B13, Bw38; Dr7) are human EBV-transformed B cell lines. Laz 509 is autologous to the T cell clones. Prior to the standard 4-hour CML assay (*3*) with ^{51}Cr-labeled Laz 156 or Laz 509 target cells, the various effector populations were incubated with one or another monoclonal antibody or with medium for 30 minutes at room temperature. The final concentrations of the antibodies were: anti-$T3_A$, 1:500; anti-$T4_A$, 1:500; anti-$T8_A$, 1:1000. (Dose-dependent blocking effects of these monoclonal antibodies were observed at concentrations between 1:100 and 1:2500). Control incubation with monoclonal antibody anti-T12 (1:500) did not influence the level of killing by the various T cell clones. The effector-target cell ratio in all experiments was 20:1. Con A was used at a final concentration of 25 μg/ml and added with the target cells at the initiation of the CML assay. This concentration of Con A has no effect on the spontaneous release of ^{51}Cr. The results are expressed as percentages of specific lysis.

Treatment	Related target (Laz 156)		Unrelated target (Laz 509)	
	Plus medium	Plus Con A	Plus medium	Plus Con A
		Clone $CT4_{II}$		
Medium	47	42	0	40
Anti-$T3_A$	12	38	0	39
Anti-$T4_A$	9	36	0	37
Anti-$T8_A$	48	44	0	41
Anti-T12	49	43	0	41
		Clone $CT8_{III}$		
Medium	68	52	0	48
Anti-$T3_A$	14	49	0	47
Anti-$T4_A$	67	52	0	49
Anti-$T8_A$	6	46	0	50
Anti-T12	67	53	0	48
		Clone $HT4_I$		
Medium	0	0	0	0

Table 2. The influence of T3 modulation on antigen-specific cytotoxicity. The $CT4_{II}$ or $CT8_{III}$ cells were incubated with monoclonal antibody to $T3_A$ (final dilution 1:300 in RPMI-1640 medium and 15 percent human serum) for 18 hours at 37°C and then washed extensively. Subsequently, untreated or T3-modulated cells were plated at an effector-target ratio of 20:1 along with ^{51}Cr-labeled Laz 156 and Laz 509 into V-bottom microtiter plates (Falcon) and then subjected to the standard 4-hour cytotoxicity assay. Con A was used at a final concentration of 25 μg/ml. The results are expressed as percentages of specific lysis.

Treatment	Related target (Laz 156)		Unrelated target (Laz 509)	
	Plus medium	Plus Con A	Plus medium	Plus Con A
		Clone $CT4_{II}$		
Untreated	47	42	0	40
T3 modulated	5	37	0	38
		Clone $CT8_{III}$		
Untreated	68	52	0	48
T3 modulated	14	49	0	46

cells with anti-T3 led to T3 antigen modulation and selective shedding of both the T3 antigen and the anti-T3 directed toward it without alterations of cell viability or changes in the density of other T cell antigens (including T1, T4, T8, T11, T12, and Ia) (*7*). These observations indicate that it should be possible to examine the lytic activity of T3-modulated cells in the absence of surface-bound monoclonal antibody that could affect steric blockade.

As shown in Fig. 1A, prior to modulation with anti-$T3_A$ all cells derived from the $CT8_{III}$ clone were reactive with anti-$T3_A$. In contrast, after $CT8_{III}$ cells were incubated with anti-$T3_A$ for 18 hours at 37°C, modulation of the T3 antigen occurred and few, if any, cells remained reactive with anti-$T3_A$ as determined by indirect immunofluorescence. Despite the loss of T3 antigen, surface expression of T8 molecules was unchanged (Fig. 1B). In a similar fashion, anti-$T3_A$ induced modulation of the T3 antigen from the T4+ clone $CT4_{II}$ without altering T4 antigen density (data not shown). After modulation with anti-$T3_A$, there was a marked reduction of lytic activity by both $CT4_{II}$ and $CT8_{III}$ (Table 2). As in the antibody-blocking experiments (Table 1), cytotoxic activity was restored in the presence of Con A and the cells did not distinguish the specific target Laz 156 from the unrelated target Laz 509. Given that this inhibition of CTL effector function occurs in the absence of residual, surface-bound monoclonal antibody, it is unlikely that the blocking effects are simply secondary to steric blockade of unrelated structures.

Similar experiments with anti-T4 and anti-T8 cannot be carried out since these antibodies do not induce modulation of their respective structures. Nevertheless, in the murine system, loss of Lyt2, the homolog of the human T8 antigen (*8*, *9*), has been induced by mutagenesis of Lyt2+ murine cytotoxic T cell clones (*10*). Such a mutation does not abrogate lytic activity but leads to loss of target specificity (*10*). These results support the view that Lyt2 and T8 are analogous and recognize similar target cell structures. The present studies also suggest that the T3 and T4 molecules on $CT4_{II}$ clones are also required for target recognition.

Since anti-T3 inhibits antigen-specific proliferation (*2*, *7*) and CTL effector function of both T4+ and T8+ clones, it is likely that T3 is linked to a nonpolymorphic portion of the receptor structures for specific antigen. That T3 is expressed on thymocytes at the time when they acquire alloreactivity and is maintained on all mature, functional T lymphocytes further supports this notion (*2*). In contrast, anti-T4 and anti-T8 each define individual subsets of human T lymphocytes and selectively inhibit the cytotoxic effector function of T4+ and T8+ clones directed at class II and class I alloantigens (*4*), respectively. Moreover, neither antibody blocks alloantigen or antigen-induced clonal proliferation.

Thus, unlike T3, T4 and T8 glycoproteins may serve as subset-restricted recognition elements in CML for individual classes of MHC gene products. In this regard, it is known that most cytotoxic T cells in mouse and man recognize antigen in the context of MHC restricting elements (*11*). Whether the occasional inability of anti-T8 (anti-Lyt2) to inhibit certain T8+ (Lyt2+) CTL effectors (*12*) is due to differential clonal T cell avidities for target antigen, specificities for non-MHC gene products, or other mechanisms of cytotoxicity (that is, natural killing) is not known.

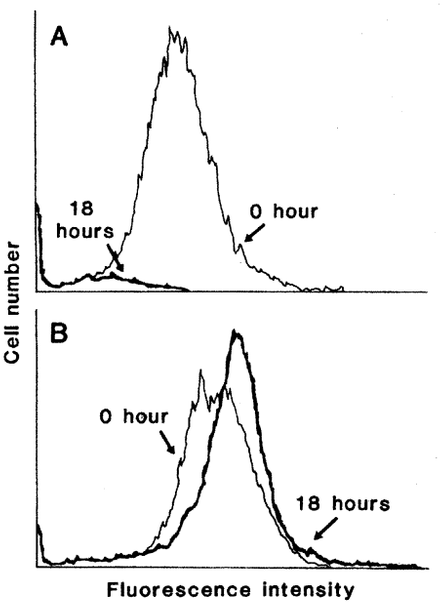

Fig. 1. Selective modulation of the T3 antigen from clone $CT8_{III}$. The $CT8_{III}$ cells were incubated with anti-$T3_A$ for 18 hours at 37°C and then washed extensively. Both untreated and T3-modulated cells (light and dark curves, respectively) were analyzed for reactivity with (A) anti-$T3_A$ and (B) anti-$T8_A$ by measuring indirect immunofluorescence on an Epics V cell sorter (Coulter Electronics) with the use of the $F(ab')_2$ fraction of goat antibody to mouse immunoglobulins conjugated with fluorescein isothiocyanate. The $T3_A$-modulated cells and the untreated cells were compared on the basis of mean channel fluorescence by using a Coulter EASY system Immuno Program (Coulter Electronics). As shown, treatment of $CT8_{III}$ cells with anti-$T3_A$ reduced anti-$T3_A$ reactivity by 96 percent, whereas anti-$T8_A$ reactivity was not diminished. In fact, the latter was slightly enhanced (105 percent) because of the additive effect of the residual anti-$T3_A$ reactivity of the modulated cells.

We suggest that structures, yet to be defined, but clearly distinct from the above surface molecules, are responsible for the lytic mechanism of CTL. Recent observations concerning high molecular weight lymphocyte surface antigens in the murine system support this notion (*13*). Functional studies with purified T3, T4, and T8 molecules derived from individual, antigen-specific T cell clones and determination of potential polymorphism of these surface structures will provide further information concerning the molecular details of T cell antigen recognition.

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