Generation of T Cells with Lytic Specificity for Atypical Antigens. III. Priming F_1 Animals with Antigen-bearing Cells also Having Reactivity for Host Alloantigens Allows for Potent Lytic T Cell Responses

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Summary

Here, we explore the conditions required for generating two different highly potent F_1 antiparental killer cell populations to unusual antigens in rats. The first, L/DA anti-DA, has lytic specificity for two antigen systems: MTA, a mitochondrial antigen expressed on DA and DA Lewis (L) target cells restricted by *RT1A* class I molecules; and H, an antigen that maps to the class I–like *RT1C* region and is present only on parental target cells from donors homozygous at the major histocompatibility complex. The second killer population is generated in the reciprocal DA/L anti-DA combination and has lytic specificity only for the H antigen system. We show that the killer cells are T cells, and that generation of these F_1 cytotoxic T lymphocytes (CTL) requires an in vivo priming step in which it is essential that the inoculated parental cells bear the relevant target antigens and possess alloreactivity for F_1 host antigens. The requirement for alloreactivity and antigen on the same priming cell population suggests that these potent lytic responses depend on a situation akin to a hapten-carrier effect that bypasses otherwise ineffective helper responses by the host to these unusual antigens.

Restimulation of F_1 lymphocytes in culture is also necessary, requiring the presence of antigen on irradiated lymphoblast stimulator cells, but alloreactivity to responder cell antigens is not necessary; normal, nonactivated lymph node cells are completely ineffective as stimulators. For effective lysis, the target cells need not possess the potential for alloreactivity to responder F_1 CTL. We also demonstrate in a preliminary way additional antigen systems defined by killer populations raised with other F_1 antiparental strain combinations.

The two previous papers in this series (1, 2) describe a pair of unusual antigen systems detectable with killer cells generated in F_1 hybrid rats against parental strain lymphoblasts. One of these antigen systems, maternally transmitted antigen (MTA),[1] is either of mitochondrial origin or depends on mitochondrial translation. Two components are required for MTA expression on target cells: a maternally transmitted factor (MTF), which is inherited extra-chromosomally and is extinguished by treatment of target cells with chloramphenicol, an inhibitor of mitochondrial translation; and $RT1A^a$ gene products of the major class I MHC locus. MTA is defined by killer cells derived from L/DA F_1 rats primed in vivo with parental strain DA lymphocytes and restimulated in culture with irradiated DA lymphoblasts. These killer cells have potent lytic specificity for target lymphoblasts of DA origin and for syngeneic DA/L F_1 lymphoblasts derived from reciprocal matings (see accompanying paper [1]).

The second antigen system, H, is unusual in two respects; its expression on target cells requires gene products of the class I–like MHC *RT1C* region, and is is also required that the *av1* allele of this locus be expressed in a homozygous manner. Gene products of the class I *RT1A* locus, the region usually associated with antigen recognition by CTL in the rat (3), are apparently not involved in the recognition of H antigen. The H antigen is detectable on DA target cells by both L/DA anti-DA and DA/L anti-DA killers raised in a manner similar to that described for MTA (see accompanying paper II [2]).

Because of the unusual nature of these two antigen systems, one of them similar in some respects to the Mta system in mice (4), but differing in its requirement for class I MHC expression, and the other requiring MHC homozygosity and expression of class I–like MHC gene products of a particular haplotype, we considered it important to define better the

[1] *Abbreviations used in this paper:* Hh, hematopoietic histocompatibility; TDL, thoracic duct lymphocytes.

conditions for generating F_1 killer cells with these lytic specificities. Previous studies of ours and others have demonstrated F_1 CTL with lytic specificity for idiotypic markers on alloreactive parental lymphocytes (5, 6). Such a circumstance, involving responder F_1 CTL and target T cells with alloreactivity towards responder CTL MHC molecules, raises the possibility that idiotype-antiidiotype mutual recognition or receptor-MHC back stimulation interactions may be important either in the generation of CTL or in the display of their lytic specificity.

In this paper, we describe the conditions for generating F_1 antiparental killer cells against these unusual antigens. We show that the killer cells are T cells and that the F_1 CTL responses require in vivo priming with antigen-bearing parental lymphocytes, and restimulation in culture with mitogen-activated parental lymphoblasts. Of particular interest, the normal parental lymphocyte populations used for priming must also contain a subpopulation reactive to host alloantigens. This appears to bypass innately weak helper cell responses in F_1 recipients that, in turn, lead to activation of unusually potent CTL responses.

Materials and Methods

All animals and procedures used in this study, except for differences noted in the text where they apply, are described earlier (see accompanying paper I [1]).

R73, a mouse IgG1 mAb for heterodimeric TCR-α/β chains of $CD4^+$ and $CD8^+$ T cells of the rat, was kindly provided by Dr. T. Hunig (7). 24B5, another mouse mAb of the same isotype, specific for phosphoryl choline (8), was used for control purposes in antibody-mediated blocking studies.

Procedures for negative selection, involving acute in vivo recirculation of parental lymphoid cells in irradiated F_1 hosts to produce populations lacking alloreactivity to selected MHC haplotypes, have been described before (9, 10).

Results

Killer Cells Specific for MTA and H Antigens Are T Cells. R73 is an IgG1 mAb that has been shown in flow cytometric studies to detect a marker coexpressed on $OX\text{-}52^+$ (pan T cell marker) CD4 and CD8 T cells of the rat (7), but not on $CD8^+$ NK cells (11); it detects a constant determinant of the rat α/β heterodimeric TCR. R73 inhibits, to a similar extent, lysis of MTA^+ DA/L target cells by L/DA anti-DA CTL (Fig. 1 *A*), lysis of H antigen–positive DA target cells by DA/L anti-DA CTL (Fig. 1 *B*), and, as expected, the lysis of DA target cells by alloreactive L anti-DA CTL (Fig. 1 *C*). Another mouse mAb of the same isotype, specific for phosphoryl choline, has no effect. We consider this result strong evidence that F_1 antiparental killer cells specific for these two atypical antigen systems are T cells.

Conditions for In Vivo Priming to Generate F_1 Antiparental CTL. The lytic activities of F_1 anti-MTA CTL (Fig. 2 *A*) and F_1 anti-H CTL (Fig. 2 *B*) were explored with F_1 lymph node cell populations that were or were not primed in vivo with DA lymphocytes. All F_1 cell populations were stimulated in vitro with irradiated DA strain lymphoblasts as described earlier (see manuscript I [1]). The results of this

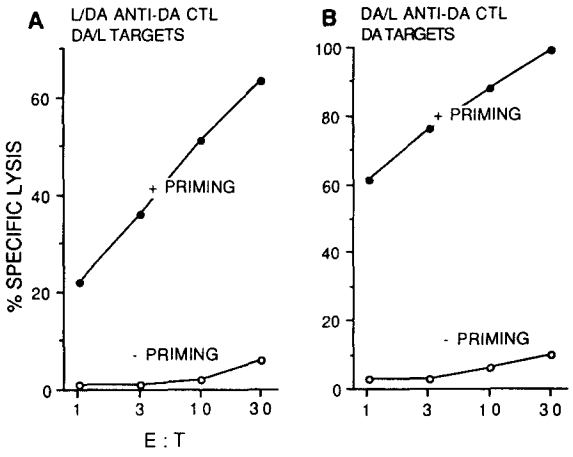

Figure 2. In vivo priming of F_1 recipients is required to generate F_1 antiparental CTL specific for both the (*A*) MTA and (*B*) the H antigen systems.

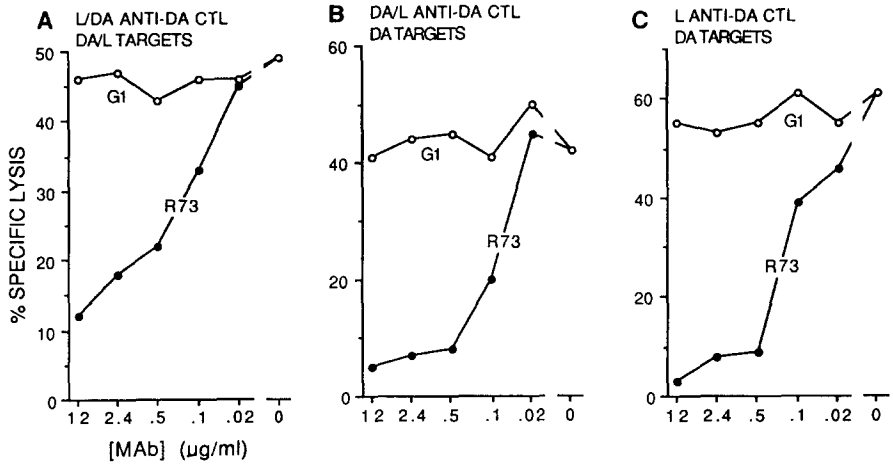

Figure 1. Evidence that F_1 antiparental killer cells are T cells. R73, an anti-rat TCR mAb blocks lysis in (*A*) the MTA system (L/DA killers on DA/L target cells), (*B*) the H antigen system (DA/L killers on DA target cells), and (*C*) a control allogeneic combination (L killers on DA target cells). G1 is an isotype control mAb.

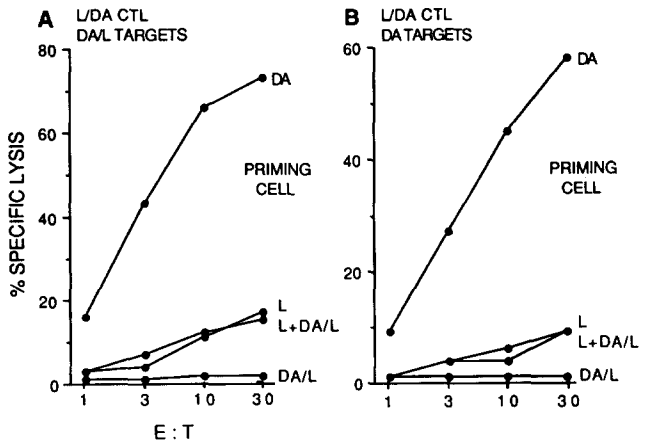

Figure 3. The presence of antigen and the potential for anti-host alloreactivity are both required of the priming cell population to generate F1 CTL with lytic effects on (*A*) MTA$^+$ DA/L target cells and (*B*) MTA/H$^+$ DA target cells. L/DA rats were immunized in vivo with various cell populations: (*a*) DA, bearing antigen and antihost alloreactivity; (*b*) L, alloreactivity alone; (*c*) DA/L, antigen alone; or (*d*) L and DA/L, alloreactivity and antigen on different cells.

experiment clearly indicate the requirement for footpad inoculation with parental cells to generate killer cells with these lytic specificities. Animals injected intravenously showed poor responses (data not shown).

Additional experiments were conducted to determine the properties of the in vivo injected parental cells required to generate F_1 antiparental CTL, specifically, whether the presence of the target antigen and/or parental cells with alloreactivity for host antigens was necessary. In this experiment,

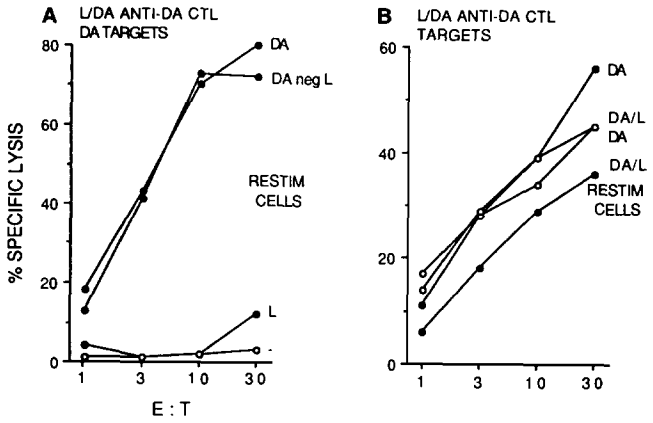

Figure 4. The cell population used for secondary in vitro stimulation to generate F_1 antiparental CTL must bear antigen, but reactivity to responder alloantigens is not required. (*A*) L/DA animals were primed in vivo as usual with DA lymphocytes, and then stimulated in vitro with (*a*) DA cells, (*b*) antigen-bearing DA_L populations negatively selected for alloreactivity to L alloantigens (DA neg L), (*c*) alloreactive parental L lymphocytes not bearing antigen, or (*d*) with nothing. (*B*) The results of a similar study using (●) MTA/H$^+$ DA target cells and (○) MTA$^+$ DA/L targets, showing that nonalloreactive, antigen-bearing DA/L F_1 cells are effective stimulators for generating L/DA CTL.

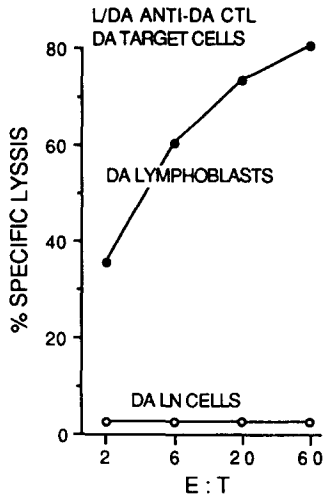

Figure 5. The restimulation of primed F_1 lymphocyte populations (L/DA anti-DA) in culture to generate F_1 antiparental CTL requires that the stimulating parental cells be previously activated; normal lymph node cells are completely ineffective.

L/DA rats were primed in vivo: (*a*) as usual with DA cells (antigen plus alloreactivity); (*b*) with L cells (alloreactivity without antigen); (*c*) with cells from recriprocal F_1 DA/L donors (antigen without alloreactivity); and with (*b*) and (*c*) above (antigen and alloreactivity on different cell populations). The results show convincingly that to generate CTL specific for MTA$^+$ DA/L targets (Fig. 3 *A*) and MTA$^+$/H$^+$ DA targets (Fig. 3 *B*) antigen or alloreactivity, alone or in combination on separate cell populations, is not adequate; these two components must be together on the same cell.

Conditions for Restimulating F_1 Antiparental CTL in Culture. Requirements for antigen and alloreactivity to generate F_1 antiparental CTL in the in vitro restimulation step were addressed in the next experiment. Cells from L/DA animals primed in vivo with DA lymphocytes were cultured (*a*) as usual with DA lymphocytes (antigen and alloreactivity); (*b*) without any further stimulation; (*c*) with L lymphocytes (alloreactivity without antigen); and (*d*) with reciprocal F_1 DA/L cells or with negatively selected parental (DA_L) lymphocytes (antigen without alloreactivity). The results of this experiment (Fig. 4, *A* and *B*) were also clear: secondary stim-

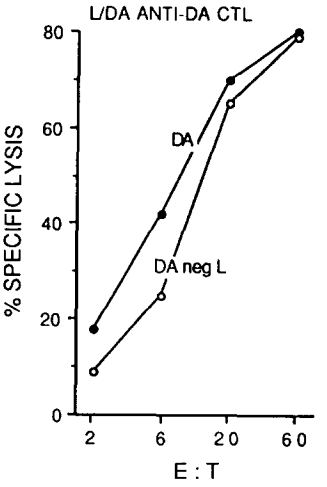

Figure 6. Effective lysis of parental cells by F_1 CTL does not require that the targets possess the potential for reactivity to killer cell alloantigens. L/DA anti-DA CTL kill DA lymphoblast targets whether or not they have been depleted of reactivity for L alloantigens (DA neg L).

ulation is necessary, and cells bearing antigen in the absence of any antiresponder alloreactive potential are sufficient.

In the next experiment, we compared normal DA lymph node cells and mitogen-activated DA lymphoblasts as in vitro stimulators of responder cells from L/DA animals primed in vivo as usual with DA lymph node cells. The results (Fig. 5) show good CTL responses after stimulation with parental lymphoblasts, and a complete absence of CTL activity after stimulation with normal DA LN cells.

For Effective Lysis by F_1 Antiparental CTL, Target Cells Need Not Be Alloreactive to the Killer Population. If alloreactivity to host antigens is required in the priming but not the restimulation step to generate F_1 antiparental CTL, the question remains whether it is a factor in the recognition by CTL of the parental T lymphoblast target cells. This appears not to be the case for MTA-specific CTL since L/DA anti-DA killers effectively lyse DA/L lymphoblasts, but it remains a potential factor in the recognition of H^+ DA target cells. An experiment to address this point was conducted by comparing the lytic efficiency of L/DA anti-DA CTL on two ^{51}Cr-labeled parental lymphoblast target cell populations: normal DA thoracic duct lymphocytes (TDL) and a population of DA TDL that had been negatively selected by acute recirculation in irradiated DA/L hosts to deplete it of anti-L alloreactivity (9, 10). The results of this experiment were clear (Fig. 6); both populations of target cells were lysed to a similar extent.

F_1 Antiparental CTL Produced in Other Strain Combinations. To obtain some estimate of the generality of the conditions described above to generate CTL populations with F_1 antiparental specificity, we conducted a limited preliminary survey of other rat strain combinations (Table 1). Some combinations (DA/WF anti-WF and WF/L anti-L) failed to generate any killer cells; four others (DA/WF anti-DA, L/WF anti-WF, WF/L anti-WF, and L/BN anti-L) showed moderate (30–39%) or potent (90–99%) lytic effects only against homozygous target cells; i.e., a pattern like the H antigen; two others (L/DA anti-L and DA/L anti-L) showed a wider pattern of lysis including significant lytic effects (20–29%) against target cells syngeneic with the killer cell population; and still one other combination (L/WF anti-L) displayed a pattern unlike the others. We have no formal evidence yet that the killer cells in these other F_1 antiparental combinations are T cells.

Discussion

An extensive literature documents the conclusion that most alloantigens that distinguish one inbred strain of animals from another or that define the uniqueness of an individual in an outbred population are defined by genes expressed in a simple codominant manner. Thus, F_1 progeny derived from interstrain matings coexpress the alloantigens of both parental strains, and consequently, for reasons of self tolerance, they are unable to react against engrafted tissues or cells of parental origin. A few apparent exceptions to this general rule, such as the H-Y antigen, determined by genes of the Y chromosome in male mice (12) and rats (3), the H-X antigen in female mice (13, 14), and the maternally inherited Mta antigen of mice (4, 15) have been noted. However, extensive analyses of these atypical antigen systems describing the genetic basis for their expression have provided an understanding for the exceptions to this general rule. The hematopoietic histocompatibility (Hh) antigen system, which involves F_1 hybrid resistance to parental marrow grafts, is another exception, but here T cells are not considered to be necessary in the rejection mechanism in vivo (16).

Table 1. *F_1 Antiparental Killer Cells Generated in Other Rat Strain Combinations*

Strain combination CTL (RT1 haplotype)	Labeled target cells										
	DA	L	L/DA	DA/L	WF	WF/DA	DA/WF	L/WF	WF/L	BN	L/BN
DA/WF (*aaav1/uuu*) anti-DA	3+	0	0	0	0	0	0	0			
DA/WF anti-WF	0	0	0	0	0	0	0	0			
L/WF (*lll/uuu*) anti-L	2+	3+	1+	0	1+	1+	0	0	0		
L/WF anti-WF	1+	0	0	0	9+	0	0	0	1+		
WF/L (*uuu/lll*) anti-L	0	0	0	0	1+	0	0	0	0		
WF/L anti-WF	0	0	0	0	6+	0	0	0	0		
L/DA (*lll/aaav1*) anti-L	1+	5+	2+	2+	2+	1+	1+				
DA/L (*aaav1/lll*) anti-L	0	4+	2+	2+	2+	1+	0				
L/BN (*lll/nnn*) anti-L	0	3+	1+				0	0		0	0

Data are summarized for E/T ratios (60:1); 0 = <10% specific lysis; each + = a 10% increment of specific lysis (i.e., 3+ = 30–39%). Designated haplotypes identify alleles at the RT1A, B/D, and C regions.

In this series of studies, we have used a somewhat unconventional means of immunizing F_1 rats with lymphoid cells of parental origin. This involves in vivo priming under GVH conditions with lymphocyte populations alloreactive to host MHC antigens, followed by in vitro restimulation with parental cells, to stimulate the development of F_1 killer T cells. These killer cells have lytic specificity for at least two, and possibly more, unusual antigen systems present on rat parental strain target cells. One of these atypical antigen systems, MTA, is similar to the mouse Mta (15) in that it appears to be of mitochondrial origin, but differs from this mouse antigen since its expression on target parental lymphoblasts depends on prototypical class I MHC gene products (see accompanying paper I [1]). The second atypical antigen, H, has an interesting property in that it requires homozygous expression of *RT1C* alleles of the class I–like MHC region of the rat (see accompanying paper II [2]).

The killer cells that detect these two antigen systems are TCR-α/β^+ T cells. This conclusion stems from the finding that lytic activity of the killer cells can be completely inhibited by R73 (Fig. 1, *A*, *B*, and *C*), a mAb specific for a constant determinant of the heterodimeric TCR-α/β molecule on OX-52^+ (pan T marker) $CD4^+$ and $CD8^+$ T cells (7). It seems likely that the blocking effects of this anti-TCR antibody are exerted on the killer cell directly, but we cannot exclude the possibility that they occur at the target cell level. If this were so, however, and if the killer cells were NK cells, it would indicate that NK cells recognize TCR molecules on target cells in this system, a possibility that has no precedent.

This paper explores the conditions and properties of parental cells used to prime and restimulate F_1 CTL that lyse MTA- and H-positive target cells. We show that in vivo priming is necessary for generating CTL for both antigen systems (Fig. 2, *A* and *B*), and for the MTA system, we show that the priming parental cell must possess both the relevant antigen and the potential for reacting to host alloantigens (Fig. 3 *A*). Priming mixtures consisting of antigen-bearing F_1 cells and lymphocytes from the other parent, lacking antigen but having alloreactivity, are ineffective. For the H antigen system, it is clear that antigen priming is necessary (Fig. 3 *B*), but we have so far been unable to assess the requirement for reactivity to host alloantigens. Priming with DA cells negatively selected for L alloantigens (DA_{-L}) causes no GVH response, and the few F_1 cells recovered from the lymph node show no proliferative activity in culture.

In vitro restimulation is necessary for generating F_1 CTL specific for these atypical antigens (Fig. 4 *A*), but here, lymphoblasts are required (Fig. 5) and it is sufficient that they express antigen only; whether they are also reactive to responder cell surface antigens is irrelevant (Fig. 4 *B*). Finally, reactivity against responder CTL appears unnecessary for the CTL-target cell interaction leading to lysis (Fig. 6).

These priming and restimulation conditions were applied to generate F_1 antiparental killer cells in a preliminary survey of nine other strain combinations. We were able to generate significant, sometimes potent, F_1 antiparental lytic activity in seven of these nine strain combinations (Table 1). None of them showed lytic specificity with a maternal effect akin to MTA. However, four displayed H-like specificity in that they effectively lysed only homozygous parental cells; two showed a wide pattern of reactivity, including specificity for what appears to be an autoantigen present on target cells syngeneic with the killer cells; and one showed a lytic pattern unlike any of the others. Several questions remain concerning the specificity of F_1 antiparental killer cells in these other strain combinations. We have yet to determine that the effector cells are T cells; it is important to determine which MHC regions are involved as targets; and it seems curious that we have found no other combinations that display MTA-like specificity. It would be of great interest to know whether the antigens detected in the F_1 antiparent responses here in the rat model are at all related to presumed Hh antigens for which a CTL model has been described in the mouse (17, 18).

The requirement that the priming cell populations used to generate F_1 antiparental CTL must also possess the potential for alloreactivity to responder alloantigens is interesting; it appears to be absolute, and it might account for the fact that CTL responses to these novel antigen systems have not been described before. Most protocols for generating CTL specific for surface alloantigens assume that these alloantigens are expressed in F_1 animals, making these unlikely responders. Despite this, it seems likely that the inability to generate these F_1 CTL unless the priming population is also reactive to host alloantigens reflects an innately inefficient host helper cell response to these antigen systems.

It has been shown in the past that CTL responses to weak antigen systems require T cell help (19–21). This can occur efficiently in the context of a hapten-carrier situation where immunizing cells carry a helper antigen in addition to the CTL target antigen (20, 22). The findings reported here, demonstrating a requirement that the priming cell possess both antigen and antihost alloreactivity, suggest a similar situation where the responding cell can supply the helper antigen.

Our provisional interpretation of this need for alloreactivity in the priming cell population is that it provides the special circumstances of linked recognition akin to a hapten-carrier effect. Parental, antigen-bearing donor T cells respond to host alloantigens in the environment of a local GVH reaction, causing the production of a variety of cytokines having potent, possibly short-range biological effects on host cells. This leads to the activation of host cells that recognize surface antigens on parental cells that are otherwise only poorly or not immunogenic. Such a model might account for the wide spectrum of autoimmune responses reported to exist in experimental and clinical settings involving GVH disease (23). It will be of interest to explore this system further for its adjuvant-like potential to generate potent immune responses to poorly immunogenic substances.

At this point we know little about the nature of these poorly immunogenic antigen systems revealed by priming under GVH circumstances. Precedent exists in both the rat and the mouse for an MHC-linked, *trans*-acting gene that imposes antigenic modifications on other MHC gene products detectable with killer T cells (24, 25); the H antigen system might be such an example. Experiments in progress indicate that in vitro stimulation of primed F_1 lymphocytes with parental cells

treated with neuraminidase to remove terminal sialyic acid residues has no effect on the specificity of antiparental CTL.

A final question concerns previous studies wherein we demonstrated that priming of F_1 animals and restimulation of their cells in culture with parental lymphocytes leads to the generation of CTL populations specific for idiotype-like specificity-associated markers on parental MLC blasts (5). We assume that subpopulations of CTL with antiidiotypic specificity exist among and are masked by the antiparental CTL populations described here, and it should be possible to reveal them at the clonal level. However, largely for technical reasons, rat CTL clones required for such studies have been difficult to produce and maintain. Recent success in establishing some cloned lines of MHC-reactive blast cells indicates that these difficulties can be overcome.

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