Numerous studies have established that murine cytotoxic T lymphocytes (CTL) recognize foreign antigens (X) in a restricted fashion (1, 2). Foreign antigens such as viral proteins, haptens, minor histocompatibility antigens, and the male specific antigen H-Y, together with products coded for by the major histocompatibility complex (MHC), contribute to the antigenic pattern recognized by CTL. It is well documented that the K, D, or L gene products of the H-2 complex are involved. However, the nature of the T cell receptor that mediates this dual specificity is still unknown. To explain the phenomenon of H-2 restriction, two models have been proposed (3). The two-receptor model postulates that CTL possess two separate types of antigen binding sites, one for X and one for self H-2 antigen. Evidence suggesting that it is the learning process during T cell ontogeny in the thymus that determines the self H-2 recognition (4, 5) strengthened this concept. The alternative model postulates that there is only one binding site for a new antigenic determinant to which both antigen X and a self H-2 antigen contribute. Adsorption experiments (6) indicate that for specific interaction between CTL and targets to occur, both antigens (i.e., self H-2 and antigen X) have to be displayed on the cell surface of targets, a result that is consistent with the latter model. So far there is no conclusive evidence that excludes either model.

The present experiments describe a different approach to the problem. Antisera were raised against virus-immune CTL in an attempt to analyze the T cell receptor by serological means, a procedure that has proved to be very useful for studying lymphocyte receptors. Anti-idiotypic (anti-id) reagents directed against cell-bound receptor molecules defined receptor idiotypes on alloreactive T cells (7, 8) and on trinitrophenyl (TNP)-specific CTL (9). Initially, it was not clear whether a similar procedure would yield anti-id sera against virus-immune CTL, mainly because it was assumed that an anti-viral CTL response might be too heterogeneous to allow the detection of such determinants on CTL generated in batch cultures. However, the present data indicate that antibodies raised in syngeneic mice recognize idiotypic
determinants on virus-immune CTL. The relevance to the two models for MHC-restricted CTL will be discussed.

Materials and Methods

Animals. CBA (H-2k), B10.ScSn (B10) (H-2b), and BALB/c (H-2d) mice were provided by the Animal Breeding Unit of the Imperial Cancer Research Fund, Mill Hill, London. B10.HTG (H-2d) mice were obtained from OLAC, Bicester, Oxon, England. In all experiments, animals of the same sex were used.

Viruses and Immunization. Newcastle disease virus (NDV) strain Ulster and influenza virus strain A/X31 were grown and titrated as described previously (10, 11). Mice were immunized with 2 × 10⁵ egg infectious dose (EID₅₀) U of NDV or influenza virus strain A/X31. Spleen cells from these donors were used as responder cells for in vitro responses. A hyperimmune anti-NDV serum was generated by injecting mice at monthly intervals with 3 × 10⁶ EID₅₀ U of NDV.

Generation of Virus-immune CTL. The method for influenza-immune CTL has been reported (12). Similarly, NDV-immune CTL were generated in vitro by culturing responder spleen cells from mice immunized with NDV 3-12 wk previously with normal syngeneic spleen cells infected with 1-2 EID₅₀ U of NDV per nucleated cell (stimulator cells). 8 × 10⁷ responder cells were cultured with 2 × 10⁷ stimulator cells in 40 ml of RPMI-1640 medium (Grand Island Biological Co., Grand Island, N. Y.) supplemented with 10% heat-inactivated fetal calf serum (FCS), 10⁻⁴ M 2-mercaptoethanol, and antibiotics. Cells were grown in tissue culture flasks (75-cm² growth area, Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, Calif.) incubating at 37°C in an atmosphere of 5% CO₂ and harvested after 5 d.

Mixed Lymphocyte Cultures (MLC). Primary MLC used splenic responder cells and gamma-irradiated (2,000 rad) allogeneic splenic stimulator cells cultured at 37°C for 5 d at a responder:stimulator ratio of 1:1.

Cytotoxicity Assays with Macrophage Target Cells. The method has been described in detail elsewhere (13). The multiplicity of infection for NDV and influenza X31 was 1 EID₅₀/cell. Targets were infected for 1.5 h at 37°C. Serial dilutions of Ficoll-Isopaque-purified CTL (14) were added after a further incubation time of 1 h at 37°C. Percent specific ⁵¹Cr release was calculated using the formula:

\[
\text{Percent Specific } ^{51}\text{Cr Release} = \left( \frac{\text{Experimental} - \text{Spontaneous Release}}{\text{Total Release} - \text{Spontaneous Release}} \right) \times 100. 
\]

Spontaneous release did not exceed 20% in any experiment and was usually <10%. Data given are the means of triplicates for assays run at 37°C for 5–8 h. SEM were usually ±3% and are omitted for clarity.

Cytotoxicity Assay Using EL4 (H-2b) BW5147 and L929 (Both H-2d) Target Cells. The method that has been described for influenza virus-infected cells (12) was also used for NDV-infected cells.

Generation and Purification of CTL for Immunization. Cultures were set up as described above, except that gamma-inactivated NDV was used (1.5 × 10⁶ rad) and cells were grown in Iscove's modified Dulbecco's medium (Grand Island Biological Co.) supplemented with normal mouse serum (NMS) from syngeneic mice instead of FCS. These mouse sera were freshly collected and heat inactivated and were used at 0.5% for CBA cultures or 1% for B10.ScSn (B10) cultures. Cells were harvested 5 later in serum-free medium, purified over Ficoll-Isopaque and washed five times in medium containing no serum.

Immunization with NDV-immune CTL. CBA and B10 mice were injected intraperitoneally with 1 × 10⁸ syngeneic NDV-immune CTL. Five injections were given at 2-wk intervals, the first in complete Freund's adjuvant, and booster injections in incomplete adjuvant. Test bleedings were carried out 10 d after the fifth injection. Sera from individual mice were heat inactivated (30 min at 56°C), absorbed twice on syngeneic spleen cells (10⁶ cells/ml antiserum), and screened for the presence of anti-id antibodies in an indirect radioimmunoassay. Mice producing anti-id antibodies were given another injection of CTL and their sera were collected 10 d later. These sera were treated as indicated above and stored in aliquots at −20°C.
Treatment of CTL with Antisera. Suspensions of CTL (4 × 10^6 cells/ml) were incubated for 45 min at 4°C in medium containing antisera (Hepes-buffered minimal essential medium supplemented with 5% heat-inactivated FCS). A monoclonal anti-Thy-1.2 (15), a hyperimmune ant-NDV serum, and anti-id sera under test were diluted to 1:500, 1:20, and 1:20, respectively. Cells were washed twice and incubated for 60 min at 37°C with a selected batch of rabbit complement at a final dilution of 1:15. Finally, cells were washed twice and tested in a ^{51}Cr release assay. Effector:target cell ratios in figures and tables are based on NMS controls.

Indirect Radioimmunoassay with CTL as Target Cells. The indirect binding assay with ^{125}I F(ab')2 sheep anti-mouse Ig (16) was adapted to virus-immune CTL as target cells. The following modifications were made. The microtiter plates (Cooke disposable flexi-plates “U” M24; Cooke Engineering Co., Alexandria, Va.) were preincubated with 150 µl FCS/well for 1 h at 37°C and then washed three times with medium. Before the test, CTL were purified on Ficoll-Isopaque. The number of cells per well was decreased to 1 × 10^4 or 5 × 10^4, and both incubations were carried out at room temperature. The input of ^{125}I F(ab')2 was 25,000 cpm/well. The medium contained 10^{-2} M NaNa. Serial dilutions of individual sera were tested. As controls, anti-H-2^b serum (D.33) or anti-H-2^k serum (D.32) from the National Institute of Allergy and Infectious Diseases, Bethesda, Md. were included in the test.

Results

Experimental System. To reduce the heterogeneity of CTL generated upon stimulation with virus-infected cells, a virus with a small number of surface proteins was selected. NDV is a paramyxovirus and codes for only two surface glycoproteins. The avirulent strain Ulster was chosen because cultured mammalian cells are nonpermissive (10) for this strain, an important prerequisite for avoiding the generation of anti-NDV antibodies in mice immunized with NDV-immune CTL.

Specificity of Virus-immune CTL. To investigate the specificity of secondary NDV-immune CTL, spleen cells from NDV-primed CBA and B10 mice were restimulated in vitro with NDV-infected syngeneic stimulator cells. These secondary effector cells fulfill all requirements for an H-2 restricted CTL response (Ursula Kees, manuscript in preparation).

Treatment of effector cells with anti-Thy-1.2 and complement abrogates their capacity to lyse syngeneic NDV-infected target cells. Fig. 1 shows the activity of B10 anti-B10 NDV CTL with or without anti-Thy-1.2 treatment assayed on NDV-infected EL4 target cells.

For lysis to occur, effector and target cells have to share the H-2K or H-2D region of the gene complex. CBA anti-CBA NDV CTL lyse NDV-infected macrophages from CBA mice, whereas allogeneic NDV-infected target cells are not recognized (Table I). Conversely, BALB/c anti-BALB/c NDV CTL lyse only syngeneic NDV-infected target cells. Thus, allogeneic NDV-infected CBA or B10 cells are not lysed, but cells from B10.HTG mice that share with BALB/c only the K and I regions of the H-2 gene complex are lysed very efficiently.

To test for virus specificity, CBA anti-CBA NDV CTL were compared with CBA anti-CBA X31 CTL. Both effector populations were assayed on NDV- and X31-infected L929 cells. The results shown in Fig. 2 indicate that these two effector populations do not cross-react, although the viruses used for stimulation are closely related.

Analysis of Anti-id Sera in Binding Tests. To raise antisera against NDV-immune CTL, a number of immunization schemes were compared. Cell doses ranged from 10^6 to 2 × 10^7 per injection. CTL were given at various intervals and by different routes of injection. Several measures were taken to reduce the generation of anti-virus
**Fig. 1.** Effect of Thy-1.2 treatment on cytotoxic activity of B10 anti-B10 NDV CTL assayed on NDV-infected EL4 target cells. CTL were untreated (---), treated with NMS and complement (---), or treated with Thy-1.2 and complement (—).

**TABLE I**

*H.2 Restriction of NDV-immune CTL Generated In Vitro*

| CTL‡ | Cytotoxicity on NDV-infected target cells* |
|------|------------------------------------------|
|      | CBA (kkk) | B10 (bbb) | BALB/c (ddd) | B10.HTG (ddb) |
| BALB/c (H.2ª) | 1:1 | 0.1 | 6.8 | 21.0 | 28.2 |
|     | 3:1 | 6.9 | 10.2 | 45.9 | 54.7 |
|     | 9:1 | 12.6 | 3.6 | 71.6 | 58.1 |
| CBA (H.2ª) | 1:1 | 13.6 | 4.7 | 0.0 | 0.0 |
|     | 3:1 | 34.6 | 8.2 | 4.3 | 11.3 |
|     | 9:1 | 49.7 | 6.0 | 5.7 | 4.7 |

* Percent ⁵¹Cr release from NDV-infected macrophage target cells over an 8-h period with release from uninfected controls subtracted. Means of triplicates are given with SEM never larger than 2.4. H.2 maps in brackets are for K, I, and D regions of the gene complex.

‡ Effector cells were generated against syngeneic NDV-infected stimulator cells and tested at the indicated effector:target ratio.

antibodies in mice immunized with CTL. For the generation of CTL, virus inactivated by gamma irradiation was used for stimulation and stimulator cells were washed three times before use in cultures. Furthermore, CTL were washed thoroughly to prevent the carry-over of virus particles into recipient mice. Positive sera were obtained as
follows. CBA and B10 mice were injected intraperitoneally with $10^7$ purified syngeneic NDV-immune CTL. Injections were given at 2-wk intervals, the first in complete Freund's adjuvant. Mice were bled 10 d after the fifth injection (see Materials and
Methods). After absorption on syngeneic spleen cells, these sera were screened for the presence of anti-id antibodies in an indirect binding assay. Analysis was carried out on NDV-immune CTL of the type used for immunization. Controls included allogeneic CTL generated against the same virus and normal spleen cells from both strains of mice. Results in Table II illustrate the binding pattern of two sera from B10 mice. Only serum B10-83 showed specific binding to B10 anti-B10 NDV CTL.

Specific binding to relevant CTL as shown in this case was obtained with very few sera. Out of 38 CBA and 44 B10 mice immunized as described above, only three mice gave a detectable response.

Functional Analysis of Anti-id Sera. Sera showing specific binding to the CTL type used for immunization were subjected to a functional test. They were assayed for their capacity to reduce the cytotoxic activity of CTL of the relevant type. Two different tests were carried out. The first involved assessment of the blocking capacity of these anti-id sera. The cytotoxicity of B10 anti-B10 NDV CTL was measured in the presence of sera B10-83 and B10-93 (both sera were positive in an indirect binding assay). Dilutions ranging from 1:10 to 1:100 did not reveal any reduction of cytotoxicity in comparison with CTL assayed in the presence of NMS (data not shown).

For the second test, CTL were treated with anti-id sera and complement. Their cytotoxicity was subsequently measured in a routine (3)Cr release assay. Cells treated with NMS and complement served as controls. Table III lists the results from three experiments. Serum B10-93 reduced the cytotoxicity of B10 anti-B10 NDV CTL by 46.5% in experiment 1 and by 66.8% in experiment 2. The cytotoxicity of CBA anti-CBA NDV CTL was not affected after treatment with this serum. Similarly, serum B10-83 consistently reacted with B10 CTL and not with CBA CTL. In contrast, serum CBA-113 had the reverse effect (experiment 3). The cytotoxicity of CBA anti-CBA NDV CTL was reduced by 91.8%, whereas no significant reduction was obtained after treatment of allogeneic CTL specific for the same virus. These results suggest that the reactivity of the three antisera tested was directed against idiotypic determinants on CTL. However, the possibility that viral determinants on CTL were recognized had to be considered. Both types of CTL used in experiments listed in Table III were generated under identical conditions and therefore have an equal

| Table II |
|------------------|
| **Binding Activity of Anti-id Sera in Indirect Radioimmunoassay** |
| | Sera‡ | Target cells* |
| | | B10 anti-B10 NDV | B10 | BALB/c anti-BALB/c NDV | BALB/c |
| Anti-H.2b | 335 ± 22§ | 350 ± 18§ | 216 ± 11 | 220 ± 16 |
| As B10-83 | 398 ± 15§ | 172 ± 11 | 220 ± 14 | 240 ± 17 |
| As B10-84 | 93 ± 18 | 139 ± 15 | 195 ± 16 | 189 ± 16 |
| NMS | 106 ± 10 | 144 ± 16 | 208 ± 19 | 203 ± 12 |

* Binding assay was carried out on 10⁶ normal spleen cells from B10 and BALB/c mice and on 10⁵ CTL generated in vitro. CTL were active in a (3)Cr release assay on syngeneic NDV-infected target cells. Data given are cpm bound (triplicates).
‡ Antisera B10-83 and B10-84 were from two B10 mice immunized with B10 anti-B10 NDV CTL. Anti-H.2b serum was serum D.33 obtained from the National Institute of Allergy and Infectious Diseases, Bethesda, Md. All sera were tested at a 1:100 dilution.
§ Significantly more binding than NMS control on the same target cells (P < 0.001)
Table III

NDV-immune CTL Treated with Anti-id Sera and Complement

| Treatment of CTL | CTL: B10 anti-B10 NDV* | CTL: CBA anti-CBA NDV |
|------------------|------------------------|-----------------------|
|                  | ^Cr release of target cells | Percent reduction | ^Cr release of target cells | Percent reduction |
|                  | B10 | B10 NDV | CBA | CBA NDV |
| Experiment 1     |     |         |     |         |
| NMS + c'         | 3.7 | 41.3    | 0   | 7.8     | 32.3 | 0 |
| As B10-93 + c'§  | NT| 22.1    | -46.5 | NT | 34.5 | +6.8 |
| Experiment 2     |     |         |     |         |
| NMS + c'         | 2.8 | 49.7    | 0   | 2.3     | 30.3 | 0 |
| As B10-83 + c'   | NT| 19.4    | -61 | NT | 36.6 | +20.8 |
| As B10-93 + c'   | NT| 16.5    | -66.8 | NT | 32.6 | +7.6 |
| Experiment 3     |     |         |     |         |
| NMS + c'         | 7.9 | 51.6    | 0   | 5.7     | 45.1 | 0 |
| As B10-83 + c'   | NT| 16.6    | -67.8 | NT | 44.1 | -2.2 |
| As CBA-113 + c'  | NT| 48.8    | -5.4 | NT | 3.7 | -91.8 |

* Effector cells were generated in vitro and tested at an effector:target ratio of 5:1.
‡ Percent ^Cr release from macrophage target cells over a 5-h period. Means of triplicates are given with SEM never >2.1.
§ All antisera were used at a 1:20 dilution.

chance of displaying viral determinants. Because sera from B10 and CBA mice reacted specifically with their relevant CTL, the likelihood that viral determinants are recognized is minimal. To further test this possibility, an additional group was included in all subsequent experiments. NDV-immune CTL were treated with a hyperimmune anti-NDV serum plus complement. No reduction of cytotoxicity was observed in any experiment, indicating that the putative anti-id sera were indeed directed toward determinants associated with the recognition site on CTL.

The results in Table III indicate that the proportion of CTL susceptible to lysis by a given anti-id serum was variable. This observation was confirmed by comparing the reduction of cytotoxicity of CBA anti-CBA NDV CTL after treatment with serum CBA-113 plus complement in three additional experiments. This serum consistently reduced the cytotoxicity of the relevant CTL. The effect, however, ranged from 45.2% reduction (experiment 2 in Table IV) to 91.8% reduction (experiment 3 in Table III). These values for the reduction of the cytotoxicity are based on the cytotoxicity measured for one effector:target ratio (see tables). A 5-h assay time for the cytotoxicity assay insures that the plateau level of ^Cr release is not yet reached.

Specificity of Anti-id Sera. To further investigate the specificity of these anti-id sera, antiserum CBA-113 was tested on a panel of CTL populations. Besides CBA anti-CBA NDV CTL (cells used for immunization), syngeneic CTL directed toward a different virus (CBA anti-CBA X31) and CTL activated in an MLC (CBA anti-B10) and (as before) allogeneic CTL directed toward the same virus (B10 anti-B10 NDV) were included. After treatment with serum CBA-113, anti-Thy-1.2, hyperimmune anti-NDV, or NMS and complement, the cytotoxicity of the four CTL populations
TABLE IV
NDV-immune CTL Treated with Anti-id Serum CBA-113 and Complement

| Treatment of CTL | CTL:B10 anti-B10 | CTL:CBA anti-CBA NDV* | Percent reduction | Percent reduction |
|------------------|------------------|-----------------------|------------------|------------------|
|                  | B10 | B10 NDV | CBA | CBA NDV |                  |
| Experiment 1     |     |         | 2.1 | 29.1 | 0 |
| NMS + c'         | 6.2 | 70.3 | NT | 15.9 | -45.1 |
| as CBA-113 + c'§ | NT | 69.6 | NT | 37.1 | -45.2 |
| Experiment 2     |     |         | 5.4 | 68.9 | 0 |
| NMS + c'         | NT | NT | NT | 15.9 | -45.1 |
| as CBA-113 + c'§ | NT | NT | NT | 37.1 | -45.2 |

* Effector cells were generated in vitro.
§ Percent 51Cr release from macrophage target cells over a 5-h period. Effector:target ratio was 4:1.
Means of triplicates are given with SEM never >2.8.
Anti-serum CBA-113 was used at a 1:20 dilution.
Not tested.

Fig. 3. Cytotoxicity of four different CTL populations tested on appropriate target cells after treatment with anti-id serum CBA-113 and complement (...), NMS and complement (--), serum CBA-113 alone (..), or left untreated (—).

was measured on their appropriate target cells. Anti-NDV serum did not have an effect on the cytotoxicity of any of the CTL populations under test, whereas treatment with anti-Thy-1.2 abrogated the cytotoxicity of all four populations (data not shown). As shown in Fig. 3A, significant reduction of cytotoxic activity was observed for CBA anti-CBA NDV CTL treated with serum CBA-113. Syngeneic CTL with different specificities (CBA anti-CBA X31 and CBA anti-B10) were not affected (Fig. 3D and
A marginally reduced cytotoxicity was measured for B10 anti-B10 NDV CTL after treatment with serum CBA-113 (Fig. 3C). Out of eight experiments carried out with serum CBA-113, this was the only one in which an effect on allogeneic NDV-immune CTL could be demonstrated.

**Discussion**

Experiments presented in this communication demonstrate that anti-id antisera can be generated against virus-immune CTL. Although antigen-binding receptors on T cells have been extensively studied on alloreactive and TNP-immune CTL (7, 8), this work has now been extended to virus-immune CTL that can be generated in vitro but that also occur naturally. The fact that specificity-associated (idiotypic) determinants can be detected on CTL generated in batch cultures indicates that the response is less heterogeneous than initially assumed. The observation that all three antisera under test reproducibly reduced the cytotoxicity of the relevant CTL by at least 45% seems to indicate that a relatively small number of different CTL clonotypes are present in batch cultures, rather than a broad spectrum of clonotypes.

The immunization protocol used here to produce antisera against virus-immune CTL is similar to the ones employed to generate antisera against alloreactive and TNP-immune CTL. The finding that the incidence of positive sera is very low is in agreement with reports from other investigators using similar procedures (17, 18). Although a number of different immunization protocols have been tested for virus-immune CTL, it may be that the optimal scheme has yet to be found. The possibility remains that recipients immunized with syngeneic CTL exercise strict regulatory control over such responses.

The three antisera shown to bind specifically to CTL used for immunization were examined for their functional properties. Blocking studies were carried out under conditions in which CTL were preincubated with anti-id antiserum, leaving the CTL in contact with the antiserum during the entire assay period. It was found that no reduction of cytotoxicity could be observed for any of the three antisera in a total of six experiments. It is possible that the titer of the antibody was not high enough for effective blocking. Furthermore, the affinity of the anti-id antibody to the receptor structure might be lower than the affinity of the receptor structure to the target cell. Similarly, unsuccessful blocking experiments have been reported using conventional antisera against T cell markers. However, in recent studies using monoclonal anti-Lyt-2 antibodies, (19) inhibition of T cell function could be shown, indicating that highly specific high titer antibodies might be required for blocking experiments.

In contrast to these blocking studies, the cytotoxicity of virus-immune CTL could be reduced by treatment of CTL with the same anti-id antiserum and complement before the cytotoxicity assay. Specificity tests carried out with a CBA antiserum (raised against CBA anti-CBA NDV effector cells) show discrimination between CTL used for immunization and syngeneic CTL generated against a different virus (CBA anti-CBA X31), or allogeneic effector cells (CBA anti-B10), as well as effector cells from H-2 different strains generated against the same virus (e.g., B10 anti-B10 NDV). This result indicates that MHC-restricted CTL carry distinct idiotypes on their surface. A suggestion for cross-reactive idiotypes was found in only one out of eight experiments, where antiserum CBA-113 reduced the cytotoxicity of B10 anti-B10 NDV CTL to a small extent.
Similar results have been reported for TNP-immune CTL where antisera raised in AKR mice could be shown to contain activity against idiotypic determinants on T cell receptors of H-2-restricted AKR anti-AKR-TNP CTL (9). It should be noted, however, that the CTL response to viral antigens can be more stringently defined as being directed against a foreign antigen in the context of self H-2. Forman and Klein (20) reported that TNP-immune CTL of wild type \( H-2K^b \) cross-react on target cells with mutant \( H-2K^{ba} \) plus TNP. This is in contrast to viral systems in which specificity is maintained between \( H-2K^b \) and \( H-2K^{ba} \) (21, 22). The cross-reactivity pattern observed for TNP-immune CTL appears to support the notion that the in vitro response to TNP self is similar to responses to allogeneic stimulators, possibly because H-2 antigens themselves are employed in the haptenation procedure (23, 24).

Based on the results presented in this report, is it possible to discriminate between a one-receptor and a two-receptor model for MHC-restricted CTL? The finding that distinct idiotypes were found on H-2-different CTL generated against the same virus is consistent with one receptor or with two closely associated receptors on CTL. In the latter case, one would have to postulate that the anti-id antibodies recognized determinants forming the junction zone between the two receptors. Because it is not known whether the postulated anti-self receptor on CTL is immunogenic, a two-receptor model cannot be excluded. However, to accommodate the reported data would mean postulating a nonoverlapping set of anti-viral receptors on H-2-disparate CTL.

Summary

Specificity-associated determinants could be demonstrated on major histocompatibility complex (MHC)-restricted virus-immune cytotoxic T lymphocytes (CTL) using antisera raised in syngeneic mice. This result indicates that the number of clonotypes in a CTL response against Newcastle disease virus (NDV)-infected syngeneic cells is sufficiently small to allow the detection of such idiotypic determinants. The functional properties of three anti-idiotypic antisera were tested in blocking studies and by measuring the reduction of cytotoxic activity of CTL after treatment with anti-idiotypic antisera plus complement. Whereas the former test did not reveal any inhibition of the T cell function, the reactivity pattern of the latter test confirmed the results obtained from binding studies, i.e., all three anti-idiotypic antisera specifically reacted with CTL of the type used for immunization and had no effect on syngeneic influenza-immune CTL, syngeneic alloreactive CTL, or NDV-immune CTL of an H-2-disparate strain. These results are discussed in terms of current models for MHC-restricted CTL responses.

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