ANTIBODIES AS ANTIGENS

The Use of Mouse Monoclonal Antibodies to Focus Human T Cells against Selected Targets

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A rationale for using mAbs in therapy is that they may act as "magic bullets" and damage the cells they bind to, either through complement-mediated cytotoxicity or the delivery of toxins or drugs (1–3). However, mAbs are not just inert messengers of destruction. They themselves behave as antigens when injected into a different species. The immune response against xenogeneic Iggs has always been regarded as an undesired side effect of mAb therapy and strategies have been devised to avoid it, such as the use of chimeric antibodies (4) or the induction of specific tolerance to foreign Ig (5).

We have investigated the possibility that the human T cell response against mouse Ig (mlg) might in some cases be beneficial, rather than detrimental, and that it could be exploited to focus an individual's T cells against targets recognized by the mAb.

Here we show that three patients who have been treated with mouse mAb carry T cells specific for mlg. These T cells are able to recognize and in some instances kill target cells that have bound and processed an antitarget mAb.

Materials and Methods

Patients. Patient A (HLA: A1, A31, B8, B37, Cw6, Cw7, DR1, DQw1) bearing a colon carcinoma was treated with a single intravenous injection (200 mg) of 17-1A mAb (IgG2a specific for an antigen associated with colon carcinoma (6) (Centocor, Malvern, PA). He neither suffered adverse reaction, nor developed detectable titers of antibodies against mouse Ig. Patient B (HLA: A1, A2, B7, B8, Cw7, DR1) bearing a colorectal carcinoma with lung metastasis was treated with three injections (once 20 mg and twice 2 mg) of 131I-F023C5 mAb (IgG1 anticarcinoembryonic antigen; Sorin Biomedica, Saluggia, Italy). He developed high titers of antibodies against mouse Ig. Patient C (HLA: A2, B35, Bw56, Cw1, Cw4, DR1, DQw1) had a B cell immunocytoma with a monoclonal IgG1λ paraprotein (1 mg/ml) in the serum. A mouse antidiotypic mAb ASI20, IgG1κ was
prepared (7). PBMC containing 40% idiotype (Id)-positive cells, were obtained before
and stored in liquid nitrogen. Large amounts of tumor cells were isolated by
leukapheresis, incubated overnight with ASI20, washed, and reinfused into the patient.
He neither showed adverse reaction nor developed anti-mIg antibodies. After treatment
3 yr ago the patient went into a slowly proceeding remission of his disease.

**Monoclonal Antibodies.** 141PF11 (IgG1 anti–human κ light chain), OKT9 (IgG1 anti-
transferrin receptor), W6/32 (IgG2a anti–human MHC class I), DA4.4 (IgG1 anti–human
IgM), BBM.1 (IgG2b anti–β2 microglobulin), 1410KG7 (IgG1 anti–human IgG), THB5
(IgG2a anti–CD-21), and 4F2C13 (IgG2a specific for an activation antigen present on
most PBMC) were obtained from the American Type Culture Collection, Rockville, MD.
SK10 (IgG1 anti–DQ), B7/21 (IgG1 anti–DP), L01.1 (IgG2a anti–transferrin receptor),
NKP15 (IgG1 anti–Fcγ receptor), and B73.1 (IgG1 anti–Fcγ receptor) were obtained
from Becton Dickinson & Co., Mountain View, CA. D4.22 (IgG1 anti–human MHC class
II) was a gift of Dr. Roberto Accolla, Ludwig Institute for Cancer Research, Lausanne,
Switzerland. The mAb 3.35 (IgG1, anti-DC-1) and its IgG2a switch variant G2a.5 were a
kind gift of Dr. Peter Parham (University of Stanford, Stanford, CA). The Ig content in
mAb preparations from ascites or culture supernatants was determined using an ELISA
assay for mouse Ig and class-matched standard Ig preparations. Purified κ light chains
were purchased from Bionetics (Charleston, SC).

**Cell Cultures.** The medium was RPMI 1640 supplemented with 2 mM L-glutamine, 5
× 10^{-5} M 2-ME, 1% nonessential amino acids, 1 mM sodium pyruvate, and 50 µg/ml
Kanamycin (complete RPMI). For the growth of human T cell clones the medium was
supplemented with 5% human serum (HS) and 30 U/ml human rIL-2 (Hoffmann-La
Roche, Basel, Switzerland). EBV-transformed B cells were maintained as described (8).

**Isolation of T Cell Clones and EBV-B Cells.** PBMC were cultured at 5 X 10^5/ml in
RPMI-5% HS in the presence or absence of different mouse mAbs. The mAbs used were:
D4.22, OKT9, and 4F2C13 at 1 µg/ml in culture. 17-IA or ASI20 were used at 1 mg/ml.
A vigorous proliferative response was observed and after 7 d the cells were expanded
with IL-2 and cloned by limiting dilution in the presence of irradiated (3,000 rad)
allogeneic PBMC, PHA (Wellcome Diagnostics, Dartford, United Kingdom, 1 µg/ml),
and IL-2 (30 U/ml) in 20 µl cultures in Terasaki trays (8). From the same three donors,
EBV-B cells were isolated and cloned as described (8). The T cell clones obtained were
screened for their capacity to proliferate in response to the mAbs used for stimulation in
the presence of autologous irradiated EBV-B cells as APCs.

**Proliferation Assay.** 2 X 10^4 T cells were cultured with 10^4 irradiated (3,000 rad) EBV-
B cells in 200 µl RPMI-10% FCS in 96-well flat-bottomed microplates in the presence or
absence of different mAbs. After 48 h the cultures were pulsed with 0.5 µCi [³H]thymidine
(sp act 5 Ci/mmol, Amersham, United Kingdom) and the radioactivity incorporated was
measured after an additional 16 h. In some experiments, EBV-B cells were pulsed with
mAbs for 30 min at 0°C, washed, and incubated at 37°C in RPMI-FCS in the presence
or absence of 10 µM chloroquine, or 5 µg/ml leupeptin (both from Sigma Chemical Co.,
St. Louis, MO). After different times of incubation at 37°C the cells were washed three
times in HBSS and fixed with 0.05% glutaraldehyde (Fluka, Buchs, Switzerland) for 1
min. The reaction was stopped by adding an equal volume of 0.2 M glycine in HBSS.
When fixed cells were used as APCs the cultures were set up in 96-well U-bottomed plates
essentially as described above.

**Cytotoxicity.** Cytotoxicity was performed as described (9). Briefly, target cells were
incubated with mAbs for 4 h at 37°C in RPMI-FCS and labeled with [¹¹⁴C]Cr. Targets (5 X
10^³) and effector cells were mixed at different E/T ratios in 200 µl RPMI-FCS in 96-well
U-bottomed microplates. The plates were incubated at 37°C and samples of supernatants
were collected after 4 h. Spontaneous, maximum (saponin), and experimental release were
measured and specific killing was determined according to the equation: Specific killing
= 100 × [(experimental release – spontaneous release)/(maximum release – spontaneous
release)].
T Cell Clones Specific for Mouse Ig Determinants

| T cell clone | [3H]Thymidine incorporated in response to:* (cpm) |
|--------------|-------------------------------------------------|
| 3.35 (IgG1)  | G2a.5 (IgG2a) Mouse κ Medium                    |
| A158         | 488                                             |
| A132         | 72,455                                          |
| B11          | 103,051                                         |
| B13          | 60,567                                          |
| C2           | 125,750                                         |
| C7           | 95,970                                          |

* Mouse Ig-specific T cell clones, originating from three different patients (A, B, and C), were cultured with autologous irradiated EBV-B cells in the presence of 10 μg/ml of the anti–DC1 IgG1 mAb 3.35, its IgG2a switch variant G2a.5, or purified mouse κ light chains. [3H]Thymidine incorporation was measured after 2 d.

Results

Patients Treated with Mouse mAbs Have T Cells Specific for mIg. PBMC from three tumor patients that had been treated with antitumor mAbs were stimulated in vitro with either high concentrations of the originally injected mAb or with low concentrations of mAbs that bind specifically to APCs, i.e., anti-human IgM or anti-MHC class II. In all three patients we observed a strong proliferative response to mIg, irrespective of the mAb used for stimulation. In contrast, normal donors did not show any detectable response. The cells that proliferated in response to mIg were almost exclusively T cells, as detected by immunofluorescent staining using anti-CD3, CD4, CD8, and WT31 mAb. In general, <10% of the blast cells were CD8+, the rest being CD4+.

The proliferating T cells from the three patients were expanded with IL-2 and cloned. 20–50% of the clones obtained were able to proliferate in response to mIg in the presence of irradiated autologous PBMC or EBV-B cells as APC. All the clones were CD3+, WT31+, CD4+, CD8- and lacked Fcy receptors as detected by the mAbs NKP15 and B73.1 (data not shown). Although we also sorted and cloned CD8+ cells, we failed to isolate CD8+ clones specific for mIg. Using as antigens a panel of mAbs of different isotypes, including switch variants as well as purified κ light chains, we found three patterns of specificity (Table I). Some clones recognized mouse IgG1, some IgG2a antibodies, and some purified κ light chains, as well as all of the κ+ mAbs tested. We conclude that in all three patients the injection of mouse mAb had induced an effective priming of CD4+ T cells against isotypic determinants of the mIg injected. An apparent exception was patient A, treated with an IgG2a mAb, from which we isolated some IgG1-specific T cell clones in addition to IgG2a specific ones. We therefore checked the isotypes present in the antibody preparation used for therapy which was prepared from ascitic fluid and found that it contained abundant amounts of mouse IgG1 in addition to the original IgG2a mAb.

We asked whether mIg, like conventional antigens, have to be processed by APCs before they can be recognized by T cells. EBV-B cells were pulsed with
Table II

**Mouse mAbs Have to be Processed by APCs before they Can Be Recognized by mlg-specific T Cells**

| APC*                          | Time at 37°C after pulsing and before fixation | $[^{3}H]$Thymidine incorporation by T cell clones: C2 | C7 | B11 |
|------------------------------|-----------------------------------------------|---------------------------------------------------|----|-----|
| EBV-B (donor C)              |                                               |                                                   |    |     |
| 0 min                        |                                               | 80 cpm                                            | 120 cpm | 128 cpm |
| 40 min                       |                                               | 118                                               | 296 cpm | 88 cpm |
| 120 min + chloroquine        |                                               | 58,273                                            | 72,125 cpm | 95 cpm |
| 120 min + leupeptin          |                                               | 1,415                                             | 2,150 cpm | ND cpm |
| EBV-B (donor B)              |                                               | 1,112                                             | 3,605 cpm | ND cpm |
| 40 min                       |                                               | 103                                               | ND cpm | 166 cpm |
| 120 min                      |                                               | 97                                                | ND cpm | 42,575 cpm |

* EBV-B cells from donor C were pulsed at 0°C with anti-IgM (DA4.4) and cells from donor B with anti-class II (D4.22) both at 10 μg/ml. After 30 min the cells were washed and incubated at 37°C for the time indicated before being fixed with glutaraldehyde. Chloroquine (10⁻⁵ M) or leupeptin (5 μg/ml) were added during the incubation at 37°C.

† T cells (2 x 10⁶) from three mlg-specific T cell clones (two from donor C and one from donor B) were incubated with fixed EBV-B cells (5 x 10⁶) in 200 μl RPMI-FCS in 96-well U-bottomed microplates. Thymidine incorporation was measured after 2 d.

anti-IgM or anti-MHC class II mAb at 0°C, washed, and incubated at 37°C for different periods of time before being fixed with glutaraldehyde. As evident from Table II, EBV-B cells that had been fixed immediately or after 40 min at 37°C were not stimulatory for T cells, while cells fixed after 120 min at 37°C were stimulatory. Furthermore, when chloroquine or leupeptin were present during the incubation at 37°C, the APC did not acquire the capacity to trigger T cells. These experiments indicate that binding of the mAb to the APC is not sufficient and that active metabolic processing events inhibitable by chloroquine (10) or leupeptin are required for effective presentation of mlg to T cells. Table II also shows that recognition of mlg requires autologous APCs, since allogeneic APCs were not effective. We therefore tried to inhibit T cell activation using anti-MHC class II antibodies. In all cases, the proliferative response to mlg could be inhibited by a rabbit anti-MHC class II antiserum (11) or by anti-MHC class II mAbs of isotypes that were not recognized by the T cell clone itself (data not shown). We conclude that mAbs, like conventional protein antigens, are recognized by T cells only in a processed form and in an MHC-restricted fashion.

Selective Presentation of mAbs that Bind to APCs. Unlike conventional antigens, mAbs can bind specifically to selected target structures on APC. We therefore investigated whether it is possible to use specific mAbs to send the same foreign antigen (mlg) to a given cell of choice so that this cell will subsequently become a selected target for mlg-specific T cells.

We therefore determined, for different mAbs, the concentration required for effective presentation to mlg-specific T cells. Fig. 1 shows the proliferative response of a T cell clone specific for mouse κ chains cultured with irradiated autologous slgM⁺-EBV-B cells in the presence of different concentrations of mAbs that either bind or do not bind specifically to the B cells. We found that antibodies that do not specifically bind to APC (anti-IgG, antiidiotype, anti-
FIGURE 1. Selective presentation of mAbs that bind to APCs. A T cell clone (C2) specific for mouse k light chains was cultured with irradiated slgM+ EBV-B cells in the presence of different concentrations of mAbs that either bind (DA4.4 [□], D4.22 [○], BBM.1 [▲], OKT9 [△], THB5 [●] and 4F2C13 [◇]) or do not bind (17-1A [■], 1410K47 [◇], AS120 [●]) to EBV-B cells. Thymidine incorporation was measured after 2 days.

colon carcinoma) were presented only at high concentrations (>10 μg/ml). In contrast, all the antibodies that bind to the B cells (anti-IgM, anti–MHC class II, anti–β2 microglobulin, anti–transferrin receptor, anti-CD21, 4F2C13) were presented to T cells at much lower concentrations ranging from 0.1 to 10 ng/ml.

Targeting T Cells Using mAbs. The above results suggest that low concentrations of specific mAbs can be used to target mIg-specific T cells against a class II–positive target of choice. We have further investigated this point by looking at whether mIg-specific T cells could be induced to selectively kill target cells that had taken up and processed a specific mouse mAb. Since processing of mIg has to take place before T cells can recognize them on APC, we first pulsed target EBV-B cells with low concentrations of mAbs for 4 h at 37°C. After labeling with 51Cr, EBV-B cells were tested as targets for lysis by autologous T cell clones specific for mIg. 8 of 20 T cell clones tested showed, in addition to a specific proliferative capacity, a strong cytotoxic activity in a 4-h 51Cr-release assay. Fig. 2 shows a representative experiment in which an mIg-specific T cell clone can efficiently lyse B cells that have been incubated with a mAb specific...
for their sIg, whereas it does not lyse B cells that have been incubated with a mAb that does not bind their sIg. We also investigated whether mIg-specific T cells are also capable of lysing bystander cells in the process of killing their specific target. For this we used as targets 51Cr-labeled EBV-B cells that had not been pulsed with the mAb, and we added, as a "triggering target," cold B cells that had been pulsed with a specific mAb. Fig. 2 shows that under these conditions there is only a limited killing of bystander cells.

The availability of an mAb specific for the idiotype of the B cell immunocytoma of patient C gave us the unique opportunity to test whether the results obtained with EBV-B cells also apply to a fresh B cell tumor. Fig. 2c shows that fresh tumor cells (of which ~40% are Id⁺) that have been pulsed with the anti-Id mAb AS120 can be killed by an mIg-specific T cell clone, while the same tumor cells pulsed with medium alone or with an irrelevant antibody are not killed. As a control, the anti-Id at the same concentration does not signal for killing an Id⁻ EBV-B cell from the same patient (Fig. 2b).

Discussion

We have found that three tumor patients treated with mouse mAbs have T cells that recognize processed mIg on autologous APC in a class II-restricted fashion, and have shown that mouse mAbs directed against various cell surface molecules can be used as antigens to focus these T cells against an MHC class II-positive target of choice.

The use of antibodies as antigens was pioneered in the mouse system by Chestnut and Grey (12), who showed that rabbit anti-mouse Ig, which binds polyclonally to B cells, is presented by B to T cells ~10⁴ times more efficiently than normal rabbit Ig. In a similar system Tony et al. (13) subsequently demonstrated that anti-MHC class I antibodies can also be presented by B to T cells, although less efficiently than anti-Ig. In the present study we have used as antigens mAbs directed against different structures on APC (sIg, class II, class I, transferrin receptor, 4F2C13 antigen), and found that all of them are presented to T cells at concentrations 10³–10⁵ times lower than those required for antibodies that do not bind to APCs. Thus many surface structures on B cells can serve for antigen internalization and class II-restricted presentation.

The rather large differences in the concentrations required for presentation among antibodies that bind to APC could be related to several factors such as: (a) the affinity of the mAbs, (b) the density of the antigen on the APCs, (c) the nature of the antigen, (d) the degree of crosslinking and (e) the rate of internalization of the complex. Indeed, when the data were normalized by relating the efficiency of presentation to the amount of mIg bound to APC at 4°C, we found that anti-Ig were presented ~10-fold more efficiently than anti-class II or anti-transferrin receptor antibodies and ~100-fold more efficiently than anti-class I antibodies (Lanzavecchia, A., and S. Abrignani, manuscript in preparation).

Targeting mIg-specific T cells with mAbs can be highly effective and specific since: (a) different molecules on the cell surface can be used as targets; (b) targeting is obtained at antibody concentrations as low as 0.1–10 ng/ml; (c) in the presence of limiting concentrations of mAbs, T cells interact only with those class II-positive cells bound by the targeting mAb. Instead, at high antibody
concentrations, the selectivity of targeting will be lost, since T cells will be distracted by antigen presented on every APC.

The limitations of antigen targeting are obviously connected with the requirement for the internalization, processing, and class II-restricted presentation of mIg. It will be important to determine: (a) whether all cell surface structures (including tumor-associated antigens) are suitable for T cell targeting by mAbs; and (b) whether class II-positive cells that are themselves neither "professional" APCs nor B cells will be able to process and present mIg to T cells.

The finding that 40% of the mIg-specific T cell clones showed, in addition to a proliferative activity, a strong MHC class II-restricted cytotoxicity, deserves some comments. The rapid cytotoxic effect and the lack of bystander lysis suggest that these T cell clones use a lytic machinery that is characteristic of typical cytotoxic T cells. However, we cannot rule out the possibility that this cytotoxic activity is acquired as a consequence of in vitro culture (14). This notwithstanding, it must be emphasized that the T cell recognition of mIg on target cells also results in secretion of lymphokines (IL-2 and IFN-γ) that have well-known immunoregulatory effects (data not shown).

The approach of using antibodies as antigens may have one advantage over that of using antibodies only as passive carriers of destructive elements: that of boosting the immune response and focusing it against the target of choice. There are examples where the response against a weakly immunogenic determinant can be elicited only if another (helper) determinant is corecognized on the same cell (15, 16). It is possible that a similar effect of "intermolecular help" may be elicited by sending the foreign antigen (mIg) to the target cell to boost the immune response against a weakly immunogenic determinant present on that same cell.

Summary

We found that three tumor patients treated with mouse mAbs have T cells that recognize processed mouse Ig on autologous APC in a class II-restricted fashion, and we have shown that mouse mAbs directed against various cell surface molecules can be used as antigens to focus these T cells on an MHC class II-positive target of choice.

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