ALLOANTIGEN RECEPTORS
ON ACTIVATED T CELLS IN MICE

I. Binding of Alloantigens and Anti-Idiotypic Antibodies
to the Same Receptors

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Antigen-specific receptors of B cells or plasma cells can easily be identified as endogenously synthesized Ig molecules. On single B cells specific Ig can directly be studied by autoradiography or immunofluorescence (1, 2), and individual Ig-secreting plasma cells can be demonstrated in a plaque assay as performed by Jerne et al. (3). The nature of the specific T-cell receptor, however, is still controversial (4). In functional tests it could convincingly be shown that T cells have antigen specificity and therefore possess specific receptors for antigens (5-8).

It would be a major advantage if one could directly study antigen-specific receptors on single T cells. To this end, Nagy et al. (9) have shown that stimulator cell alloantigens were present on the cell surface of mixed lymphocyte reaction (MLR) activated responder T-cell blasts. The binding of alloantigens was specific in the sense that responder T-cell blasts stimulated by two histogenetically different stimulator cells bound either one or the other type of stimulator cell antigen. This indirectly demonstrated the presence of a specific T-cell receptor for alloantigens. The same group established that alloantigen binding was dependent on active biosynthesis of T-cell receptors by the responder T-cell blasts (10).

In a different experimental system, anti-idiotypic antibodies against idiotypic determinants on T cells specific for histocompatibility antigens have been used as a probe for T-cell receptors. Such anti-idiotypic antibodies have most successfully been induced in rats against antihistocompatibility antibodies as well as against parental T cells undergoing a graft vs. host reaction (GvH) in F1 hybrid animals (11, 12). It has been difficult to use these procedures for raising similar anti-idiotypic antibodies in mice (13). Using MLR-selected T-cell blasts as antigens in F1 hybrid mice, however, we found satisfactory titers of circulating anti-idiotypic antibodies.

Binz and Wigzell (12) showed that treatment of MLR responder cells with anti-idiotypic antibodies and complement diminished the ability of these cells to proliferate against the relevant stimulator cells. It has not been formally

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1 Abbreviations used in this paper: Con A, concanavalin A; FCS, fetal bovine serum; GvH, graft vs. host reaction; HBSS, Hank's balanced salt solution; Hepes, N-2-hydroxyethylpiperazine-N'-2-ethane sulfonic acid; LD, lymphocyte-defined MLR-stimulating determinants; MLR, mixed lymphocyte reaction; MLR-activated T-cell blasts, (B6)AKR-T, (SJL)AKR-T, (B6)SJL-T, (B6)(AKR × SJL)FI-T, and (SJL)(AKR × B6)FI-T; stimulator strain in the first, MLR-activated responder T-cell blasts in the second position; PBS, phosphate-buffered saline; RaT, rabbit anti-mouse T-cell serum; SI, stimulation index.
demonstrated, however, that the anti-idiotypic antibodies react with the receptor molecules for alloantigens on T cells. In this paper, therefore, we studied T-cell receptors for alloantigens by alloantigen binding and idiotypic analysis. In particular, we tested whether anti-idiotypic antibodies and alloantigens compete for sites on the receptor molecules on T cells. Our results suggest that the same receptor molecules are identified by either reagent.

Materials and Methods

**Animals.** AKR/J (H-2k), C57Bl/6 (H-2b), SJL/J (H-2s), and F1 hybrid mice between AKR/J and B6, and AKR/J and SJL/J were used.

**Media.** The culture medium for MLR was (10 mM) N-2-hydroxyethylpiperazine-N'-2-ethane sulfonic acid (Hepes)-buffered RPMI-1640 (Grand Island Biological Co., Grand Island, N. Y.) supplemented with L-glutamine (2 mM final concentration), streptomycin (100 μg/ml), 5% human serum (a pretested batch which allowed high stimulation in MLR and kept the [³H]thymidine uptake in syngeneic MLR cultures low), and 2-mercaptoethanol (3 × 10⁻³ M final concentration). The medium used for the preparation of cell suspensions and washing of cells was the same except that it did not contain 2-mercaptoethanol. The concentration of human serum in this medium was 2.5% (wash medium).

Immunofluorescence was performed in Hanks' balanced salt solution (HBSS) with 10% fetal calf serum (FCS) (heat inactivated) and 10⁻² M Na-azide.

**Cell Preparation for MLR Cultures.** Lymph node cells from axillary, cervical, inguinal, and mesenteric nodes were prepared as described previously (14). The cells were counted and viability was determined by exclusion of trypan blue 0.16% in saline. Responder cells were enriched for T cells by passing normal lymph node cells over nylon wool columns. Greater than 99% of the nonadherent cells were Ig-negative (as tested by immunofluorescence; between 38% and 71% of the cells loaded on the column were recovered).

**Nylon Wool Column Procedure.** The method was a modification of that described by Julius et al. (15). 10-ml plastic Luer lock syringes were densely packed with 1.6 g nylon wool (LP-1 Leukopak filter; Fenwal Inc., Walter Kidde & Co., Inc., Ashland, Mass.) and sterilized by autoclaving. Before use, the columns were washed with 50 ml phosphate-buffered saline (PBS) and 10 ml PBS containing 5% heat-inactivated FCS. The column was then incubated for at least 1 h at 37°C. After incubation, the column was washed with 50 ml PBS containing 5% FCS (37°C). Up to 1.25 × 10⁸ lymph node cells in 2 ml of PBS containing 5% FCS were applied to the column. 2 ml of PBS containing 5% FCS were used to wash the cells farther into the column (drop by drop). After 45 min of incubation at 37°C, the nonadherent cells were washed out of the column with 50 ml of prewarmed (37°C) wash medium.

**MLR.** Unidirectional MLR cultures were set up in the following way. Stimulator cells were X-irradiated (3,300 rad). The responder cells were nylon wool column-separated lymph node cells. The concentration of responder cells was adjusted to 5 × 10⁶ cells/ml, that of stimulator cells to 1 × 10⁷ cells/ml. 2 ml of responder cell suspension was mixed with an equal volume of stimulator cell suspension (ratio 1:2) in a Falcon 30-ml plastic bottle (no. 3012; Falcon Plastics, Div. of BioQuest, Oxnard, Calif.). The bottles were incubated in an upright position in a 5% CO₂–95% air humidified atmosphere for 4 days. After 3 days, 40 μCi of [³H]thymidine (2 Ci/nmol; The Radiochemical Centre, Amersham, England) was added and thymidine incorporation was determined on day 4 of culture. The stimulation index was calculated as the ratio of counts in alloimmune cultures to counts in syngeneic cultures.

**Mitogen Activation.** Spleen cells (5 × 10⁶ cells/ml) in the same medium as that used for MLR cultures were stimulated for 2 days with 5 μg or 10 μg concanavalin A (Con A; Boehringer/Mannheim, no. 15237) per ml medium. [³H]Thymidine incorporation was measured after a 24-h labeling period.

**Preparation of Blasts.** Blasts were recovered from MLR cultures on day 4 by centrifuging 5 ml of cultured cells over a 4-ml Ficoll-Urovison layer (density 1.077 g/cm³; Schering AG, Berlin, West Germany) and resuspended in 12-ml glass tubes at 600 g for 15 min at room temperature. Cells (at least 98% viable) were recovered from the interface and washed three times in medium. Usually, >85% of the viable cells obtained from the allogeneic cultures were large- and medium-
sized blasts. Blast cells were defined as cells with a diameter at least twice that of a small lymphocyte, with a smaller nuclear-to-cytoplasmic ratio than small lymphocytes, and with a nonsegmented nucleus (as distinct from nonlymphoid cells).

**Alloantigen Binding Assay.** The method used to detect specific rebinding of alloantigens to trypsinized T-cell blasts was similar to that described by Elliott et al. (10). The following steps were sequentially undertaken to perform the complete assay: (a) isolation of MLR-activated T-cell blasts; (b) identification of these blasts as responder T cells with bound stimulator cell alloantigens; (c) trypsin treatment of T-cell blasts. The T cell blasts were washed twice in RPMI-1640 with 0.03 M Hepes and 5% heat-inactivated FCS (Gibco Diagnostics, Chagrin Falls, Ohio), counted, washed once more in RPMI-1640 without serum or Hepes, and resuspended in HBSS to a concentration of 2 × 10^6 cells/ml. To 0.2-1 ml of each cell suspension, an equal volume of PBS containing 5 mg/ml tosyl-L-phenylalanyl-chlormethane-treated trypsin (Merck AG, Darmstadt, West Germany) was added, and the mixture was then incubated at 37°C for 30 min. To stop the reaction, ice-cold RPMI-1640 (10 ml) with 15% FCS was added; the cells were counted, and washed three times. To enable them to recover from trypsin treatment, the T-cell blasts were cultured at a concentration of 5 × 10^6 cells/ml overnight at 37°C, in 5% CO₂, and in a humidified atmosphere in fresh culture medium in Falcon flasks (model 3012); (d) rebinding of alloantigens to trypsin treated blasts. After overnight incubation, the trypsin-treated cells were counted, washed once, and resuspended to 1.5 × 10^6 viable cells/ml; 0.3-ml aliquots of this cell suspension were incubated for 5 h with 0.7 ml of fresh tissue culture medium, or medium containing alloantigen (stimulator cell material). Afterwards, the cells were counted again. Allogeneic cell material bound by the recovered responder T-cell blasts was detected by indirect immunofluorescence after the cells had been incubated first with antistimulator alloantisera, and then with fluorescent anti-mouse Ig antibodies. As a source of stimulator cell material, we used the supernate from 4-day MLR cultures collected after centrifuging the whole culture twice at 400 g for 10 min.

**Competition of Alloantigens and Anti-Idiotypic Antibodies for Receptors on MLR-Activated T Cell Blasts.** MLR-activated T-cell blasts were incubated overnight after trypsin treatment with MLR supernates (as a source of alloantigens) from allogeneic or syngeneic MLR cultures, as described above. Indirect fluorescent staining of the recovered blasts with anti-idiotypic serum and fluorescent anti-mouse Ig antibodies (see below) was performed the next day.

**Hyperimmune Alloantisera.** AKR anti-B6, AKR anti-SJL, and B6 anti-AKR were raised, absorbed, and tested for specificity as previously described (9, 16).

**Normal Mouse Sera.** Normal mouse sera used were a pool of serum from 20-30 mice. All sera were heat inactivated (56°C, 30 min).

**Rabbit Anti-Mouse T-Cell Serum (RaT).** RaT serum was a gift from Dr. C. Bron, Institut de Biochimie, Université de Lausanne, Switzerland (17).

**Anti-Idiotypic Sera.** The principle of the production of anti-idiotypic sera was the injection of isolated MLR-activated T-cell blasts into F₁ mice (crosses between responder and stimulator strains). This is illustrated by the following example. The isolated (B6) AKR-T cell blasts were washed five times in 10 ml HBSS and injected intraperitoneally into 13 (AKR x B6)F₁ hybrid mice. Each mouse was given three injections of isolated T-cell blasts: (a) 1 × 10⁶ cells in complete Freund's adjuvant; (b) 5 × 10⁵ cells in incomplete Freund's adjuvant (1-wk interval); (c) 3.5 × 10⁶ cells in incomplete Freund's adjuvant (2-wk interval). The sera used in our experiments gave essentially the same results. They were a pool of a bleeding of all 13 (AKR x B6)F₁ hybrid mice obtained 2 and 3 wk after the last injection, respectively. The sera will be referred to as F₁ anti-(AKR anti-B6) sera, in short F₁a(AKRαB6).

**Absorption of Anti-Idiotypic Sera.** 50 μl of the sera (at a predetermined dilution in HBSS) was mixed with 8 × 10⁶ T-cell blasts resuspended in a known volume of HBSS. This mixture was incubated for 30 min on ice, the cells were spun down for 10 min at 16,000 g, and the serum was recovered. The dilution was then calculated. This procedure was repeated twice.

**Absorption of F₁a(AKRαB6) Serum on Alloantisera.** 0.8 ml each of normal AKR serum, and hyperimmune AKR anti-SJL, and AKR anti-B6 sera were couples to 2.7 g (wet weight) cyanogen bromide-activated Sepharose 4B (Pharmacia Inc., Piscataway, N.J.) according to the method described by March et al. (18). 100 μl of (SJL)AKR-T cell blast-preabsorbed F₁a(AKRαB6) serum (see above) was absorbed for 1 h at 4°C with 100 μl of packed, coupled Sepharose. The absorbed F₁a(AKRαB6) serum was recovered by centrifugation of the mixture at 16,000 g for 10 min.
Special care was taken that the absorbed sera had exactly the same dilution when used to stain (B6)AKR-T cell blasts in indirect immunofluorescence.

**Immunofluorescence.** The cells were washed three times in fluorescence medium at 4°C, and were kept throughout in this medium. They were resuspended in 100 μl of medium to which 100 μl of the appropriate fluorescent reagent was added (at a predetermined dilution), incubated at 4°C for 30 min, and washed three times between each incubation. After the final antiserum treatment, the cells were washed three times and mounted on glass slides: the preparations were scored under a Zeiss fluorescence microscope (Photomik III; Carl Zeiss, Inc., New York).

Ig+T+ blasts were detected by treating the cells simultaneously with rabbit anti-mouse T-cell serum (dilution 1/400) and the appropriate mouse antiserum was detected by tetramethylrhodamine isothiocyanate-conjugated sheep anti-rabbit IgG and fluorescein isothiocyanate-conjugated sheep anti-mouse IgG. The anti-Ig sera were raised and conjugated with fluorochromes as described (19), with the modification that in all cases only fluorescent anti-Ig antibodies were used which were purified by affinity chromatography on Ig columns (20) before coupling with fluorochromes. All sera as well as all fluorescent conjugates were centrifuged at 16,000 g for 10 min immediately before use.

**Results**

**Specific Rebinding of Alloantigens to MLR-Activated T-Cell Blasts after Removal of Cell Surface-Bound Stimulator Cell Alloantigens by Trypsin Treatment.** Specific rebinding of stimulator cell alloantigens to trypsin-treated, MLR-activated responder T-cell blasts after recovery from trypsin treatment is shown in Table I. The data in this table are from one representative experiment out of three which gave essentially the same results. We used AKR as responders and B6 and SJL as stimulators in MLR, as these strains show a large number of histocompatibility differences (21). Trypsin treatment and overnight incubation in MLR culture medium (without alloantigens) of MLR-activated AKR responder T-cell blasts reduced the number of these cells which could be stained with responder anti-stimulator serum to a level below 5%. 52% of (B6)AKR-T cell blasts bound B6 and only 10% SJL alloantigens; this was significantly above background staining with normal mouse serum instead of alloantisera. (SJL)AKR-T cell blasts revealed the reverse specificity, i.e. 57% of these blasts bound SJL and only 14% bound B6 alloantigens. Control staining with normal mouse sera instead of alloantisera gave fewer than 3% Ig+T+ blasts.

**Reactivity of Anti-Idiotypic Antibodies with MLR- or Mitogen-Activated T Cells.** As shown above, a large number of (B6)AKR-T cell blasts had B6 alloantigens bound, possibly to receptors on the cell surface. We therefore raised anti-idiotypic antibodies by injection of these blasts into (AKR × B6)F1 hybrid mice. We used this combination to avoid production of alloantibody (anti-B6 antibodies). These antibodies could in principle be generated if syngeneic (i.e. AKR) mice were used as recipients.

With the F1a(AKRA) sera normal B6, (AKR × B6)F1, and (AKR × SJL)F1 lymph node T cells did not stain above background in comparison to controls with normal (AKR × B6)F1 serum. Conversely, normal AKR lymph node T cells stained minimally above background. An exact estimate of the precursor frequency of idioype-positive AKR T cells, however, cannot be given with the immunofluorescent method due to the difficulty in establishing the significance of a low number of positive cells above background.

The results from Table II demonstrate that the majority (65%) of (B6)AKR-T
### Table 1

**Specific Rebinding of Alloantigens from MLR Supernates to MLR-Activated Trypsinized T-Cell Blasts after Recovery from Trypsin Treatment**

| Responder | Stimulator | Trypsinized T-cell blasts incubated with supernates from MLR | Alloantisera for detection of bound alloantigens | Ig-positive responder T-cell blasts$\S$ |
|-----------|------------|-------------------------------------------------------------|--------------------------------------------------|-----------------------------------|
| AKR       | B6         | (i) AKR                                                     | B6                                               | AKR anti-B6 52                    |
| AKR       | B6         | (ii) AKR                                                    | SJL                                              | AKR anti-SJL 10                   |
| AKR       | SJL        | (iii) AKR                                                   | SJL                                              | AKR anti-SJL 57                   |
| AKR       | SJL        | (iv) AKR                                                    | B6                                               | AKR anti-B6 14                    |

* Responder cells were nylon wool column-passaged nonadherent lymph node cells (over 99.5% Ig-negative by immunofluorescence; 57% recovery of cells from the nylon wool column). AKR(R), B6(S): SI 515; AKR(R), SJL(S): SI 184. 
† Both alloantisera were hyperimmune sera, used at a final dilution of 1/10, and absorbed once with AKR spleen cells. Akr anti-B6 serum was absorbed three times with SJL and AKR anti-SJL serum three times with B6 spleen cells. 
§ Ig$^+$T$^+$ blasts were detected by indirect immunofluorescence (see Materials and Methods), they were over 99% T cells and over 97% responder cells (staining with B6 anti-AKR serum). <5% of trypsinized T cell blasts were Ig positive with alloantisera after overnight incubation in culture medium. Background staining with normal mouse serum was <3%. Between 100 and 300 T-cell blasts were counted. 
|| Percent recovery of cells and percent blasts after overnight recovery from trypsin treatment in culture medium and 5 h incubation with MLR supernate. Recovery: 90% (i); 100% (ii); 40% (iii); 75% (iv); blasts: 84% (i), 74% (ii), 68% (iii), 63% (iv). 

and only 5% of (B6)SJL-T-cell blasts stained with the F$a(AKR_aB6)$ serum. The latter number of positive blasts was possibly within the background even though normal (AKR × B6)$_F$ serum gave only 1% positive T-cell blasts. To test whether other AKR T-cell blasts reacted with the antisera, we performed immunofluorescent staining with Con A-stimulated AKR spleen cells. 7.9% of the Con A-stimulated splenic T-cell blasts were positive. This was within the control with normal (AKR × B6)$_F$ serum of 8.2%. 

Further specificity tests also showed that 36% of (SJL)AKR-T-cell blasts were positive (32% in a separate experiment). Our initial assumption was that lymphocyte-defined MLR-stimulating determinant (LD) antigens on SJL public with B6 could lead to an expansion of AKR responder T-cell clones with common idiotypes. To test this hypothesis, we absorbed the F$a(AKR_aB6)$ serum with an equal number of either (B6)AKR-T or (SJL)AKR-T cell blasts, respectively, and obtained the following data from immunofluorescent staining with the absorbed antisera: (B6)AKR-T-cell blast-absorbed antisera stained only 8.6% of (B6)AKR-T-cell blasts, whereas (SJL)AKR-T-cell blast-absorbed antisera still stained 35.1% of (B6)AKR-T, but only 5.3% of (SJL)AKR-T-cell blasts. These data would be compatible with the above assumption. 

Reactivity of F$a(AKR_aB6)$ Serum with MLR-Activated F$_1$, Hybrid T Cells. The crucial test of whether or not public LD determinants on B6 and SJL could lead to stimulation of some of the same AKR T cells and hence to expression of the same receptors and idiotypes on these cells is in the staining
T-CELL RECEPTORS FOR ALLOANTIGENS

### Table II

**Reactivity of Anti-Idiotype Serum with MLR- or Mitogen-Activated T Cells**

| Cell population | Immunofluorescence with Fα(AKRaB6) serum* | Ig-positive responder T-cell blasts† |
|-----------------|------------------------------------------|------------------------------------|
| **Responder**   | **Stimulator**                           | %                                  |
| AKR             | B6                                       | Unabsorbed                         | (i) 65.0±8.6                        |
|                 |                                           | Absorbed on (B6)AKR-T cell blasts‡ | 8.6                                |
|                 |                                           | Absorbed on (SJL)AKR-T cell blasts‡ | 35.1                               |
| AKR             | SJL                                      | Unabsorbed                         | (ii) 36.0±5.8                       |
|                 |                                           | Absorbed on (SJL)AKR-T cell blasts‡ | 5.8                                |
| SJL             | B6                                       | Unabsorbed                         | (iii) 5.0±1.7                       |
| BJ              |                                           | Con A-activated splenic T-cell blasts | Unabsorbed | (iv) 7.9±1.7 |

* The antiserum (unabsorbed and absorbed) was used in a final dilution of 1/20.
† Between 100 and 300 blasts were counted.
‡ Responder cells were nylon wool column-passaged nonadherent lymph node cells (over 99.5%
Ig-negative by immunofluorescence; between 38% and 48% recovery of cells from the nylon
wool column). AKR(R), B6(S): SI 616, over 99% blasts. AKR(R), SJL(S): SI 306.5, 97% blasts,
over 99% responder T cells (staining with RaT and B6 anti-AKR serum). SJL(R), B6(S): SI
8.5, 91% blasts (over 99% T cells).
§ Range in three experiments: 65%–84% (i), in two experiments; 32%–36% (ii). Normal (AKR ×
B6)F1 serum control: 1.2% (i); 0.7% (ii); 1% (iii); and 8.2% (iv).
¶ 50 μl of the antiserum was absorbed twice on 8 × 10^6 MLR-activated T-cell blasts.
** Activation for 2 days by 5 μg and 10 μg Con A/ml medium. Blasts were pooled from both
cultures and identified as T cells by RaT. SI: 24.5 (5 μg culture), 20.8 (10 μg culture). 90%
blasts.

The fact that not only the majority of (B6)(AKR × SJL)F1-T cell blasts but also a large number of (SJL)(AKR × B6)F1-T-cell blasts were positive with the antiserum (Table III) does not favor our assumption. Possibly, other as yet unidentified antibodies contaminate the antiserum and are mainly responsible for staining of (SJL)AKR-T and (SJL)(AKR × B6)F1-T cell blasts. Data from Table III also show that these contaminating antibodies can be removed by appropriate absorptions without affecting activity against idiotype-positive T-cell receptors for B6 antigens. Absorption of the Fα(AKRaB6) serum with (B6)AKR-T and with (SJL)AKR-T cell blasts, respectively, equally removed the activity of the antiserum against (SJL)(AKR × B6)F1-T-cell blasts. In marked contrast,
Reactivity of Anti-Idiotypic Serum with MLR-Activated F₁ Hybrid T Cells

### Table III

| Cell population         | Immunofluorescence with F₃a(AKRaB₆) serum* | Ig-positive responder T-cell blasts |
|-------------------------|-------------------------------------------|-----------------------------------|
|                         |                                           | %                                 |
| T-cell blasts from MLR§ |                                           |                                   |
| Responder Stimulator    |                                           |                                   |
| (AKR × SJL)F₁ B6        | Unabsorbed                                | (i) 81%                           |
|                         | Absorbed on (B6)AKR-T-cell blasts         | 6.7                               |
|                         | Absorbed on (SJL)AKR-T-cell blasts        | 33                                |
| (AKR × B6)F₁ SJL        | Unabsorbed                                | (ii) 56%                          |
|                         | Absorbed on (B6)AKR-T-cell blasts         | 11                                |
|                         | Absorbed on (SJL)AKR-T-cell blasts        | 11                                |

* For absorption of the antiserum see Table II. The antiserum was used in a final dilution of 1/15 on (B6) (AKR × SJL)F₁-T and 1/100 on (SJL) (AKR × B₆)F₁-T cell blasts.
† Between 100 and 300 blasts were counted.
§ Responder cells were nylon wool column-nonadherent lymph node cells (over 99% Ig-negative by immunofluorescence; recovery of cells from nylon wool column: 40% (AKR × SJL)F₁, 64% (AKR × B₆)F₁, (AKR × SJL)F₁(R), B₆(S): 98% blasts. (AKR × B₆)F₁(R), SJL(S): 84% blasts.
‖ Normal (AKR × B₆)F₁ serum control: negative (i), and 5% (ii).

Reactivity of the antiserum with (B₆)(AKR × SJL)F₁-T cell blasts could be removed only by absorption with (B₆)AKR-T, and not with (SJL)AKR-T-cell blasts. The latter absorption, however, reduced the number of positive (B₆)(AKR × SJL)F₁-T-cell blasts from 81% to 33%.

Competition of Alloantigens and F₃a(AKRaB₆) Antibodies for Binding Sites of the Same T-Cell Receptors. In several experiments we had observed that the idiotypic determinants recognized by the F₃a(AKRaB₆) serum were removed from the T-cell blast surface by trypsin treatment and resynthesized after overnight incubation of the trypsinated blasts in culture medium. In addition, the immunofluorescent staining on a large fraction of the recovered blasts seemed to be more intense than on the freshly isolated blasts. Therefore, we performed experiments (a representative example is shown in Table IV) to test whether there could be competition (or at least steric hindrance) of alloantigens and F₃a(AKRaB₆) antibodies for binding sites of T-cell receptors for alloantigens, i.e. whether alloantigens and anti-idiotypic antibodies bind to the same receptor molecules. Idiotype-positive receptors and bound B₆ alloantigens were demonstrated on (B₆)AKR-T-cell blasts [68% F₃a(AKRaB₆) and 53% B₆ positive]. Trypsin treatment reduced the staining in both cases to background levels. After overnight incubation of the trypsinated blasts in tissue culture medium (without soluble alloantigens), most of the blasts stained very strongly with the F₃a(AKRaB₆) serum. Bound B₆ alloantigens could not be shown [71.8% F₃a(AKRaB₆) and 2.7% B₆ positive].

If, however, the trypsinated blasts were incubated overnight in an MLR supernate which contained B₆ alloantigens [MLR:AKR(R), B₆(S)], a large
number of the recovered (B6)AKR-T-cell blasts had B6 alloantigens bound, whereas staining with F,\alpha(AKRaB6) serum was markedly reduced [9% F,\alpha(AKRaB6) and 50% B6 positive].

To establish the specificity of this inhibition, we tested whether or not two other control MLR supernates could inhibit the reactivity of the F,\alpha(AKRaB6) serum with the (B6)AKR-T-cell blasts. These control supernates were from the MLR: (a) AKR(R), AKR(S) (as a source of AKR antigens); and (b) AKR(R), SJL(S) (as a source of SJL alloantigens). In both cases, staining with the anti-idiotypic serum was only minimally reduced [54.5% and 44.5% F,\alpha(AKRaB6) positive, respectively]. Only 5.4% and 11.8%, respectively, of the (B6)AKR-T-cell blasts stained for crossreacting alloantigens with AKR anti-B6 serum. The number of idiotype-positive (B6)AKR-T-cell blasts in the latter two cases was not drastically lower than the number after incubation of the blasts in culture medium alone. The inhibition of the staining of F,\alpha(AKRaB6) serum on (B6)AKR-T-cell blasts mainly with B6 alloantigens was specific, as staining with rabbit anti-mouse T-cell serum (RaT) serum on the same blasts was not depressed. Soluble B6 alloantigens and F,\alpha(AKRaB6) anti-idiotypic antibodies thus seem to bind to the same receptor molecules.

Reactivity of F,\alpha(AKRaB6) Serum with AKR Anti-B6 Antibodies. (SJL)AKR-T-cell blast-absorbed F,\alpha(AKRaB6) serum proved to be specific for receptors on (B6)AKR-T and (B6)(AKR × SJL)F,\alpha-T-cell blasts. To test whether or not this antiseraum would also specifically react with AKR anti-B6 alloantibodies, it was used for immunofluorescent staining of (B6)AKR-T-cell blasts after absorption on AKR anti-B6 serum-coupled Sepharose. Control stainings were done with F,\alpha(AKRaB6) serum absorbed on normal AKR or AKR anti-SJL serum. It can be seen from Table V that only absorption on AKR anti-B6 serum-coupled Sepharose significantly reduced the number of stainable (B6)AKR-T-cell blasts—by 75% (from 46.4% to 11.3% Ig-positive responder T-cell blasts). This indicates that at least a fraction of AKR anti-B6 antibodies share common idiotypic determinants with T-cell receptors of (B6)AKR-T-cell blasts.

Discussion

The experiments reported in this paper demonstrated the presence and specificity of T-cell receptors for alloantigens on MLR-activated responder T-cell blasts detected by the binding of soluble stimulator cell alloantigens and anti-idiotypic antibodies to the same receptor molecules.

In accordance with Elliott et al. (10) we could show that soluble alloantigens from MLR supernates specifically bind to responder T-cell blasts after recovery of these cells from trypsin treatment, i.e. the number of (B6)AKR-T-cell blasts binding B6 alloantigens was greater than five times the number binding SJL alloantigens. The reverse was seen for (SJL)AKR-T-cell blasts.

Nevertheless, a small but significant number of (B6)AKR-T- and (SJL)AKR-T-cell blasts bound SJL and B6 alloantigens, respectively. This binding was detected with AKR anti-SJL and AKR anti-B6 sera even after extensive absorption for reactivity with public B6 or SJL SD determinants. AKR anti-SJL serum therefore reacted with private SJL SD determinants on SJL alloantigens bound by a small number of (B6)AKR-T-cell blasts, whereas AKR anti-B6
**Competition of Alloantigens and Anti-Idiotypic Antibodies for Binding Sites of T-Cell Receptors**

* Ig-positive immunofluorescent (B6)AKR-T cell blasts*  

**TABLE IV**  

**Reactivity of Anti-Idiotypic Serum with Alloantibodies**

| Cell population | T-cell blasts from MLR§ | Immunofluorescence with Fₐa(AKRaB6) serum* | Ig-positive responder T-cell blasts† |
|-----------------|------------------------|---------------------------------------------|------------------------------------|
| Responder       | Stimulator             |                                              | %                                  |
| AKR             | B6                     | Unabsorbed                                  | 46.3                               |
| AKR             | B6                     | (i) Absorbed on normal AKR serum∥           | 46.4                               |
| AKR             | B6                     | (ii) Absorbed on AKR anti-SJL serum∥        | 39.7                               |
| AKR             | B6                     | (iii) Absorbed on AKR anti-B6 serum∥        | 11.3                               |

* The antiserum used in all four groups was preabsorbed on (SJL)AKR-T cell blasts as described in Table II and used at a final dilution of 1/18.  
† Between 150 and 300 blasts were counted.  
§ AKR responder cells were nylon wool column-nonadherent lymph node cells (over 99.5% Ig-negative by immunofluorescence; recovery of cells from nylon wool column: 45%). AKR(R), B6(S): SI 65.6.  
∥ The preabsorbed (see *) Fₐa(AKRaB6) serum was further absorbed on normal AKR serum (i), hyperimmune AKR anti-SJL (ii), or AKR anti-B6 serum (iii) coupled to cyanogen bromide-activated Sepharose as described under Materials and Methods.

serum reacted with private B6 SD determinants on B6 alloantigens bound by a small number of (SJL)AKR-T-cell blasts.  
These data can probably be explained by the assumption that alloantigens from MLR supernates are part of complex membrane fragments. Thus, private
SD determinants recognized by our absorbed alloantisera could physically be linked with public determinants recognized by T-cell receptors for alloantigens. This interpretation necessarily implies that alloantibodies and T-cell receptors recognize different public histocompatibility determinants.

With respect to B6 and SJL alloantigen binding by (B6)AKR-T and B6 and SJL alloantigen binding by (SJL)AKR-T cell blasts, the question arises whether this binding is due to crossreactivity of the T-cell receptors on single cells from a small fraction of MLR-activated T-cell blasts, or whether two different types of receptors are expressed on separate clones. The observation that responder T-cell blasts from an MLR with two histogenetically different stimulator cells in the same culture bind either one or the other type of stimulator cell alloantigen (9) rather favors the concept of separate clones.

In addition to alloantigen binding, we studied T-cell receptors for alloantigens with anti-idiotypic sera. Since it has been difficult to raise such antisera in mice with alloantibodies as immunogens, or with parental T cells undergoing a GvH response in F1 hybrid mice (13), we tried to raise anti-idiotypic sera by repeated injection of purified MLR-activated responder T-cell blasts into F1 hybrid mice. As the majority of MLR-activated responder T-cell blasts invariably carry stimulator cell antigens bound to receptors on the cell surface (see above), F1 hybrid mice (crosses between the MLR responder and stimulator strains) were used as recipients. Thus, we tried to avoid alloantibody production against stimulator alloantigens, which could have occurred with syngeneic recipients. Syngeneic recipients for the production of anti-idiotypic sera against MLR-activated T-cell blasts were used by Andersson et al. (22). This provides the advantage of testing whether or not the T-cell response of these mice is specifically suppressed (tested by MLR) against cells from the MLR stimulator strain. This system however, suffers from the disadvantage that a suppressive role of alloantibodies against stimulator cells cannot easily be excluded.

In addition, these authors had generated the MLR-activated T-cell blasts by an MLR with an unseparated responder cell population containing T and B cells. We could previously show (9) that the resulting T-cell blasts from such an MLR have alloantibodies on the cell surface. These alloantibodies, produced by coproliferating responder B cells, are bound to the cell surface of responder T-cell blasts through a bridge of stimulator cell alloantigens specifically attached to the alloantigen receptors of these responder T-cell blasts. The use of such blasts therefore could lead to the production of a mixture of anti-idiotypic antibodies which might react with T-cell receptor idiotypes as well as with idiotypes of alloantibodies. We wanted to avoid this situation by injecting MLR-activated responder T-cell blasts from an MLR with nylon wool column-purified lymph node T cells as responders into F1 hybrid mice (crosses between MLR responder and stimulator strains). Our method of raising anti-idiotypic sera has by now been successfully reproduced by various experimental groups. That is, F1a(AKRaSJL) sera gave results similar to F1a(AKRaB6) sera, but with the reverse specificity.

In this paper we reported that F1a(AKRaB6) sera have high titered antibodies against idiotypic determinants of T-cell receptors from (B6)AKR-T and (B6)(AKR × SJL)F1-T-cell blasts against B6 alloantigens. This observation is supported by the high number of these T-cell blasts which reacted with
F, α(AKRaB6) sera, and by the fact that absorptions of the sera with (SJL)AKR-T-cell blasts did not abolish the reaction of the antisera with (B6)AKR-T and (B6)(AKR × SJL)F1-T-cell blasts. Furthermore, the reaction of the antisera with these blasts was specific, as (B6)SJL-T- and Con A-activated splenic AKR T-cell blasts did not react with the antisera above background.

Nevertheless, unabsorbed F, α(AKRaB6) sera also reacted with some (SJL)AKR-T and (SJL)(AKR × B6)F1-T-cell blasts. This reactivity could in both cases be absorbed out equally well with (B6)AKR-T as with (SJL)AKR-T-cell blasts. These data strongly suggest that, together with anti-idiotypic antibodies against AKR or (AKR × SJL)F1 receptors for B6 antigens exclusively, other as yet unidentified contaminating antibodies are present in these antisera. Such antibodies could be directed against viral determinants (16) on the T-cell blast surface; they could be autoantibodies, antibodies against blast antigens, or antibodies against human serum components. We have excluded a major role for autoantibodies by concluding that F, α(AKRaB6) sera did not react with normal B6, (AKR × B6)F1, or (AKR × SJL)F1 lymph node T cells (data not shown) and normal AKR lymph node T cells stained only minimally above background (data not shown). A high titer of possibly contaminating antibodies against blast antigens and human serum components eventually picked up by the T-cell blasts from the MLR culture medium is equally unlikely, as the F, α(AKRaB6) sera did not stain (B6)SJL-T- and Con A-activated splenic AKR T-cell blasts above background.

Initially, we assumed that F, α(AKRaB6) sera apart from (B6)AKR-T also stained a fraction of (SJL)AKR-T-cell blasts because public SJL LD determinants with B6 might have stimulated the expression of receptors on (SJL)AKR-T-cell blasts with idiotypic determinants shared by receptors on some (B6)AKR-T-cell blasts. Even though raised against (B6)AKR-T-cell blasts, F, α(AKRaB6) sera would recognize these common idiotypes on both types of T-cell blasts. This assumption, however, was not supported by the fact that F, α(AKRaB6) sera also stained a sizable number of (SJL)(AKR × B6)F1-T-cell blasts. In this situation only private SJL and not public SJL LD determinants with B6 could have been responsible for MLR stimulation. If our explanation had been correct, (SJL)(AKR × B6)F1-T-cell blasts should have been negative with the antisera.

Alternatively, cross-reactive idiotypes could be expressed on a fraction of (B6)AKR-T-, (B6)(AKR × SJL)F1-T-, (SJL)AKR-T-, and (SJL)(AKR × B6)F1-T-cell blasts; F, α(AKRaB6) sera might recognize idiotypic determinants on receptors from MLR responder cells against either modified self antigens (23) or blast antigens presented by MLR stimulator cells which escaped irradiation.

Another set of data bears on the question of allelic exclusion. F, α(AKRaB6) sera stained (B6)AKR-T- but not (B6)SJL-T-cell blasts. This excludes common idiotypes on both types of blasts. Surprisingly, however, the majority of (B6)(AKR × SJL)F1-T-cell blasts were positive. This might be explained either by the absence of allelic exclusion or by the rather unlikely assumption that in this MLR, only (AKR × SJL)F1 T-cells with AKR-type receptors responded to B6 stimulators.

As discussed above, alloantigen binding and idiotypic analysis provide two independent probes for T-cell receptors on MLR-activated T-cell blasts. We
combined these two approaches to test whether or not anti-idiotypic antibodies and alloantigens compete for sites on the receptor molecules on T cells.

We could show that idiotype-positive T-cell receptors on (B6)AKR-T-cell blasts could be resynthesized after overnight incubation and recovery from trypsin treatment as demonstrated by staining with F1a(AKRaB6) serum. Uptake of B6 alloantigens to (B6)AKR-T-cell blasts after trypsin treatment, however, could effectively block the staining of (B6)AKR-T-cell blasts with F1a(AKRaB6) serum. This inhibition was specific, as demonstrated by lack of considerable inhibition with control alloantigens. Alloantigens and anti-idiotypic antibodies therefore seem to bind to the same receptor molecules on MLR-activated T-cell blasts.

The fact that AKR anti-B6 serum-coupled Sepharose could specifically absorb out a considerable part of the reactivity of F1a(AKRaB6) serum with (B6)AKR-T-cell blasts indicates that at least a fraction of alloantibodies share idiotypic determinants with receptors on alloreactive T cells. Whether some or all of such idiotypic determinants on T-cell receptors are identical to idiotypic determinants on alloantibodies can be studied in a radioimmune assay with F1a(AKRaB6) serum and purified radioactive alloantibodies. Such work is in progress in our group.

Our data provide some information on the specificity of T-cell receptors for alloantigens. Alloantigen binding and idiotypic analysis of T-cell receptors with antisera raised against idiotypes on receptors of MLR-activated responder T-cell blasts make possible the direct study of T cells on a single-cell level. We showed that it is possible reproducibly to raise anti-idiotypic sera in mice. These sera, however, have to be vigorously controlled for specificity. The specificity of the antisera can be assessed as reported in this paper. We obtained further specific controls by functional studies, such as direct MLR inhibition by addition of anti-idiotypic sera to the culture. Data from these experiments support the findings in this study and will be reported elsewhere.

We shall concentrate our future work with these antisera on the genetics of T-cell receptor idiotypes, and the estimation of the precursor frequency of alloreactive T cells. The latter question could not be approached effectively with the fluorescent methodology in this study due to inherent problems with background levels which do not allow correct measurements of low numbers. Finally, the anti-idiotypic sera shall provide an excellent tool to screen T-cell tumors or T-cell tumor/T-cell hybrids for expression of receptors for alloantigens.

Summary

B6 alloantigens in supernates from one-way mixed lymphocyte reaction (MLR) cultures of AKR T cells against B6 lymph node cells rebound specifically to (B6)AKR-T-cell blasts after overnight incubation and recovery of these blasts from trypsin treatment. A similar specificity was observed with the binding of SJL alloantigens to (SJL)AKR-T-cell blasts. In both cases, the corresponding alloantigens were rebound several-fold more efficiently than control alloantigens. In a different assay system, T-cell receptors were studied with anti-idiotypic sera. These antisera were raised by repeated injection of purified (B6)AKR-T-cell blasts into (AKR × B6)F1 hybrid mice. These F1a(AKRaB6) sera reacted
with the majority of (B6)AKR-T and (B6)(AKR × SJL)F₁-T-cell blasts. They also reacted with a lower, though sizable, number of (SJL)AKR-T- and (SJL)(AKR × B6)F₁-T cells. No reaction was observed with (B6)SJL-T, concanavalin A-activated AKR T-cell blasts, normal B6, (AKR × B6)F₁, and (AKR × SJL)F₁ T cells. Normal AKR T cells were positive only minimally above background. Fₐ(AKRaB6) sera could be made specific for (B6)AKR-T and (B6)(AKR × SJL)F₁-T-cell blasts by absorption of contaminating antibodies with (SJL)AKR-T-cell blasts.

Finally, it was shown by competition experiments that the receptors on MLR-activated T-cell blasts that bind alloantigens were the same as those binding anti-idiotypic antibodies. In addition, it was found that at least a fraction of alloantibodies share common idiotypic determinants with receptors on MLR-activated T-cell blasts.

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