SPECIFICITY OF T CELL CLONES FOR ANTIGEN
AND AUTOLOGOUS MAJOR HISTOCOMPATIBILITY COMPLEX
PRODUCTS DETERMINES SPECIFICITY FOR FOREIGN
MAJOR HISTOCOMPATIBILITY COMPLEX PRODUCTS*

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The high frequency of T lymphocytes recognizing allelic variants of major
histocompatibility complex (MHC) products has puzzled immunologists. More
than 50%, and possibly all T cells, recognize products of foreign MHC genes (1–3).
Further studies have indicated that T cells specific for complexes of conven-
tional antigen and self-MHC products (4) also react with foreign MHC products
(5–8). This has suggested that a high degree of homology between (a) complexes
composed of an individual’s own MHC products and antigen, and (b) foreign
MHC gene products, is responsible for alloreactivity. Another possibility is that
receptors for foreign MHC products are distinct from receptors for complexes
formed by an individual’s MHC and foreign proteins (9).

The first demonstration that a single clone of T cells could recognize autolo-
gous class II MHC products associated with antigen as well as foreign class II
MHC products, came from a reaction of a T cell clone with specificity for (a)
ovalbumin plus syngeneic I-Ak gene products and (b) I-Ak gene products (10). A
study of a series of BALB/c antiovalbumin clones also indicated that several
reacted to foreign MHC products, and that these reactions were randomly
distributed among the foreign MHC haplotypes tested (11). However, neither
observation indicated whether or not “alloreactivity” included serum proteins
in culture and, more importantly, whether this was due to binding by its receptor
for conventional antigen and autologous MHC products.

A direct approach to this requires a panel of cloned T cells with virtually
identical specificities for a well-defined antigen and a particular self-MHC prod-
uct. We shall term this physiologic reaction corecognition. If alloreactivity is, in
fact, generated as a direct consequence of the gene product(s) responsible for
corecognition, this panel should display a pronounced bias of reactivity to a
particular foreign MHC product. We have tested this hypothesis and the results
reported here indicate that a substantial number of clones with indistinguishable

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Abbreviations used in this paper: DME, Dulbecco’s modified Eagle’s medium; FCS, fetal calf serum; 2-ME, 2-mercaptoethanol; MHC, major histocompatibility complex.
fine specificities for foreign insulin associated with the I-A\textsuperscript{b} gene product also react with a particular and unique foreign MHC gene product.

**Materials and Methods**

***Mice.*** C57BL/10, B10.A, DBA/1, ILn/J, PL/J, SJL/J, RI11/J, SM/J, and B6AF\textsubscript{1} mice were obtained from The Jackson Laboratory, Bar Harbor, ME. Females between the ages of 6 and 10 weeks were used. C3H.LG and B10.PL mice were kindly donated by Dr. M. Dorf, Pathology Department, Harvard Medical School.

***Antigens and Immunizations.*** Crystalline cow, sheep, and pig insulins were the generous gifts of Dr. R. E. Chance of the Lilly Research Laboratories, Indianapolis, IN. B chain of cow insulin was purchased from Sigma Chemical Co., St. Louis, MO.

***Antibodies.*** The following affinity-purified monoclonal antibodies were generously donated by Dr. J. Freed and Dr. J. Kupinski, Johns Hopkins University School of Medicine: anti-I-A\textsuperscript{a} 10-3.6, 0.6 mg/ml; anti-I-E\textsuperscript{a} 14-4-4S, 1 mg/ml; anti-I-E\textsuperscript{a} 17-3-3S, 1 mg/ml; anti-I-A\textsuperscript{b} 25-9-17, 1 mg/ml. Anti-I-A\textsuperscript{b} culture supernatants 28-16-8 and 25-9-3 were the kind gifts of Dr. D. Sachs, National Institutes of Health.

***Preparation of conditioned medium.*** T cell growth factor was prepared by sequential treatment of B6AF\textsubscript{1} spleen cells with neuraminidase and galactose oxidase according to the method of Novogrodsky and Katchalski (12). Briefly, spleen cell suspensions are incubated at a concentration of 80 × 10^6 cells/ml in 0.1 IU/ml neuraminidase (Calbiochem-Behring Corp., La Jolla, CA) for 30 min at 37°C, washed with phosphate-buffered saline and suspended in 10 U/ml galactose oxidase (Millipore Corp., Freehold, NJ) at a final concentration of 20 × 10^6 cells/ml for 30 min at room temperature. The cells were then washed and resuspended at a final concentration of 5 × 10^6 cells/ml in (Dulbecco's modified Eagle's medium (DME) (Gibco Laboratories, Grand Island, NY) containing 4% fetal calf serum (FCS), 2 mM L-glutamine (Gibco Laboratories) and 5 × 10^{-5} M 2-mercaptoethanol (2-ME). Supernatants were collected 18-24 h later, centrifuged, filtered and stored at −20°C until use.

***Development and Maintenance of Antigen-specific Clones.*** 2 wk after immunization of mice with cow or sheep insulin, draining lymph nodes were removed. Cell suspensions were incubated in 25-cm\textsuperscript{2} flasks (3012; Falcon Labware, Oxnard, CA) at a concentration of 4 × 10^6 cells/ml in Click’s medium supplemented with 0.5% normal mouse serum, 4 mM L-glutamine, 2.5 mM sodium pyruvate (M.A. Bioproducts, Walkersville, MD), 5 × 10^{-5} M 2-ME, and 100 μg/ml of the insulin used for immunization. 7 d later, 10^5 viable cells were incubated with 5 × 10^5 syngeneic irradiated (2,000 rad) spleen cells supplemented with 10% conditioned medium, 5% FCS, 4 mM L-glutamine, 5 × 10^{-5} M 2-ME, and 100 μg/ml insulin in a Linbro well (Flow Laboratories, Inc., Hamden, CT). Each week, an aliquot of 10^5 cells was removed and incubated with fresh irradiated spleen cells in cDME; antigen was added every other week. After 4-6 wk of growth, cells from these lines were cloned by limiting dilution. Cells were seeded into microtiter wells (Falcon 3040) containing 10^6 irradiated feeder cells and 50 μg/ml antigen, in cDME. Within 10-14 d, growth was visible in 50% of wells seeded at 0.5 cell/well; the cloned lines were expanded in Linbro wells and maintained in the same manner as the parent cell line. Cloned lines are supplemented every 2-3 d with cDME containing 5-10% conditioned medium. Clone C5, a C57BL6 anti-3-nitro-4-hydroxyphenyl acetyl (NP) clone, was donated by C. Clayberger (Dana-Farber Cancer Institute) for this study.

***Assay for Antigen-specific \(^{3}H\) Thymidine Incorporation.*** 7-14 d after stimulation with fresh irradiated (2,000 rad) spleen cells with or without added antigen, the cloned cells were harvested, washed, and resuspended (10^4/0.2 ml) in DME containing 5% FCS, 4 mM L-glutamine, 5 × 10^{-5} M 2-ME and 2.5 mM sodium pyruvate in microtiter wells containing 5 × 10^5 irradiated (2,000 rad) spleen cells with or without different antigens. Unless otherwise specified, 1 μCi/well [\(^{3}H\) thymidine was added after 24 h and the cells harvested 16 h later using a MASH (Belco Glass, Inc., Vineland, NJ). Data is expressed as mean cpm incorporated ± standard deviation of triplicate wells. Inhibition of proliferation by monoclonal antibodies directed against various I region products of the MHC was assayed after addition of antibody at initiation of the proliferative assay.
Results

10 different T cell clones were initially tested for alloreactivity against a panel of nine MHC different target cells (Table I). Clones BC4, BC6, BC8, and BC10 were derived from a single cloning of a C57BL/10 (B10) anti-cow insulin cell line. Clone C5 is a C57BL/6 anti-NP clone. Clone AS7 is a B10.A anti-sheep insulin clone. Clones AS6, AC4, AC7, and AC8 are all B10.A derived and are reactive to syngeneic (B10.A) spleen cells in the presence of FCS. Three B10 anti-cow insulin clones reacted to PL/J (H-2^b) target cells. The same three (out of four) clones that reacted to PL/J also showed a small, but significant, response to SM/J cells of the H-2^d haplotype.

Clones BC4, BC6, BC8, and BC10 all react to cow insulin and not to sheep or pig insulin (Fig. 1). Since cow and sheep insulins differ from each other at only a single residue, position 9 of the A chain, the specificity of these clones can be localized to the alpha loop of the insulin molecule. Moreover, all of these clones co-recognize cow insulin associated with I-A^b subregion products (not shown); thus, all four display identical or very similar antigen specificity.

Because all four of these clones were derived from a single cell line, we could not exclude the possibility that BC4, BC6, and BC8 were daughter cells of one cell in the line that also, by random chance, reacted to PL/J. Therefore, we raised a second independent line of cow insulin specific cells from B10 mice, and derived a second panel of clones from this cell line. Results of this cloning are shown in Table II. Of seven clones raised from B10 mice immune to cow insulin, six reacted with cow and not with any other insulin, and are thus specific for the alpha loop of cow insulin. Of these six clones, two were alloreactive to PL/J spleen cells but not to any other foreign haplotype tested (not shown). The seventh clone, which is specific for B chain determinants of native insulin, showed no alloreactivity. An aliquot of cells from this same initial noncloned population was stimulated in vitro with the isolated B chain of cow insulin. All of the clones derived were specific for the denatured B chain of cow insulin (4/4), and none were reactive to PL/J or to any other MHC haplotype tested. Other B10 cells were selected for reactivity to sheep insulin. These were specific for the B chain.

Table I

| Targets* | Proliferative responses (\(^{3}H\)thymidine incorporation) of clones† |
|----------|--------------------------------------------------------------------------------|
|          | BC4 | BC6 | BC8 | BC10 | C5 | AS6 | AS7 | AC4 | AC7 | AC8 |
| C57BL/10† | 464 | 373 | 402 | 750  | 646| 171 | 810 | 652 | 1,655| 765 |
| B10.A     | 345 | 239 | 254 | 251  | 505| 130 | 90,767| 47,920| 74,898| 75,570|
| DBA/1     | 1,259| 954 | NT | 598  | NT | 810 | 829 | 486 | 575  | 558 |
| RII       | 975 | 600 | NT | 536  | NT | 205 | 601 | 1,017| 67   | 126 |
| SJL       | 1,317| 965 | NT | 1,026 | NT | 847 | 925 | 740  | 1,030| 430 |
| L/Lo      | 539 | 644 | 305 | 1,172 | NT | 270 | NT  | NT  | NT   | NT  |
| C3H.LG    | 1,299| 628 | NT | 1,304 | NT | 1,108| 1,102| 737  | 951  | 771 |
| PL/J      | 37,719| 49,119| 75,599| 1,198| 672 | 257 | 1,484| 276  | 1,201| 574 |
| SM/J      | 3,705| 4,425| 9,707| 1,287 | NT | 295 | NT  | NT  | NT   | NT  |
| Syngeneic plus antigen | 50,445| 54,399| 116,527| 21,446| 12,857| 76,505| —   | —   | —   | —   |

* 5 x 10^5 irradiated (2,000 rad) spleen cells.
† 2 x 10^4 cloned T cells were used per well. Numbers represent the mean of triplicate cultures. Standard errors have been omitted for the sake of simplicity; all were <10% of the mean.
‡ H-2 haplotypes are, respectively, b, a, q, r, s, j, f, u, and v.
§ Not tested.
FIGURE 1. B10 anti-cow insulin clones: $10^4$ cells from each of four clones from the B10 anti-cow insulin cell line were assayed for specificity and H-2 restriction. Either medium or 100 $\mu$g/ml cow, sheep, or pig insulin was added. $5 \times 10^5$ irradiated (2,000 rad) syngeneic (B10) or allogeneic (B10.A) feeder cells were used. $[^3]$H]thymidine uptake was measured on day 2.

**TABLE II**

Specificity and Alloreactivity of T Cells Derived from Second Cloning

| Clone | $[^3]$H]thymidine incorporation* in response to: | B10 spleen cells† plus: |
|-------|---------------------------------------------|---------------------|
|       | B10 spleen cells† plus:                     | PL/J                | B10.A   |
|       | Medium | Cow insulin | Sheep insulin | B chain | PL/J | B10.A |
| BCC4  | 378    | 40,317      | 3,277        | 1,994   | 47,111 | 258    |
| BCC7  | 2,647  | 53,297      | 2,998        | 3,681   | 23,019 | 450    |
| BCC8  | 869    | 80,872      | 3,875        | 1,730   | 653    | 312    |
| BCC11 | 4,066  | 72,179      | 5,475        | 2,985   | 479    | 419    |
| BCC12 | 257    | 14,913      | 296          | 239     | 257    | 156    |
| BCC13 | 184    | 4,857       | 276          | 235     | 267    | 150    |
| BCC14 | 478    | 11,818      | 14,797       | 14,286  | 431    | 251    |
| BCB1  | 2,229  | 3,732       | 2,555        | 128,643 | 262    | 167    |
| BCB2  | 315    | 1,410       | 790          | 43,997  | 293    | 1,351  |
| BCB4  | 399    | 463         | 838          | 9,011   | 460    | 137    |
| BCB6  | 250    | 374         | 244          | 6,240   | 263    | 248    |
| BSS2  | 1,101  | 17,631      | 70,294       | 79,473  | 271    | 206    |
| BSS3  | 1,515  | 19,115      | 59,059       | 62,183  | 261    | 207    |

* Means of triplicate cultures are shown. Standard errors are <10%.
† $5 \times 10^5$ irradiated (2,000 rad) spleen cells were used per well, with medium or 100 $\mu$g/ml of antigen.
‡ From $10^4$ to $2 \times 10^4$ cloned T cells were cultured in each well.
of native insulin; none reacted with PL/J (or with other MHC haplotypes tested). A summary of these data is presented in Table III: 23 clones were tested for alloantigen recognition; 5 of 10 clones specific for the alpha loop of cow insulin reacted with PL/J cells. None of the remaining 13 clones that displayed related but not identical antigen specificities reacted with PL/J cells.

To confirm that these reactions were properties of a single cell, one of the

| Specificity                          | Number PL/J-reactive clones/total |
|-------------------------------------|-----------------------------------|
| B10 anti-cow insulin (A chain)      | 5/10                              |
| B10 anti-cow insulin (B chain)      | 0/1                               |
| B10 anti-sheep insulin (B chain)    | 0/2                               |
| B10 anti-B-chain (denatured)        | 0/4                               |
| B6 anti-NP                          | 0/1                               |
| B10.A anti-sheep insulin (A chain)  | 0/1                               |
| B10.A anti-'B10.A'                  | 0/4                               |

**Table IV**

**Antigen Specificity and Alloreactivity of BC4 Subclones**

| Clone         | [3H]thymidine incorporation in response to: | [3H]thymidine incorporation in response to: |
|---------------|---------------------------------------------|---------------------------------------------|
|               | B10* + cow insulin§                        | PL/J*                                       |
| Parent BC4    | 51,313 ± 275                                | 40,719 ± 225                                |
| BC4.BC.E9     | 32,254 ± 2,829                              | 24,382 ± 1,407                              |
| BC4.BC.D7     | 33,069 ± 572                                | 25,395 ± 1,209                              |
| BC4.BC.D5     | 52,880 ± 854                                | 41,094 ± 572                                |
| BC4.BC.D11    | 18,126 ± 613                                | 12,827 ± 244                                |
| BC4.BC.F8     | 49,828 ± 1,279                              | 41,481 ± 521                                |
| BC4.PL.C9     | 23,542 ± 458                                | 19,774 ± 390                                |
| BC4.PL.C5     | 54,534 ± 4,585                              | 31,844 ± 3,906                              |
| BC4.PL.C11    | 53,074 ± 6,969                              | 38,476 ± 1,818                              |
| BC.PL.D7      | 18,870 ± 572                                | 16,960 ± 1,209                              |

* 5 x 10⁵ irradiated (2,000 rad) spleen cells were used per culture.
§ 100 μg/ml insulin. Proliferation in the absence of insulin for all subclones is <100 cpm.
* Clones labeled BC4.BC. were subcloned on irradiated B10 spleen cells and cow insulin at 0.3 cells/well.
§ Clones labeled BC4.PL. were subcloned on PL/J spleen cells at 0.3 cells/well.

**Table V**

**Requirement for a Non-MHC Antigen of PL/J in Alloreaction**

| Clone | [3H]thymidine incorporation in response to:* |
|-------|---------------------------------------------|
|       | C57BL10 | B10.PL | PL/J   |
| BC4   | 4.196 ± 500 | 736 ± 768 | 175,121 ± 1,014 |
| BC6   | 481 ± 92  | 812 ± 379 | 51,499 ± 7,287  |

* 2 x 10⁶ cloned T cells were incubated with 5 x 10⁵ irradiated (2,000 rad) spleen cells from the indicated strains for 40 h.
**TABLE VI**

*Fine Specificity of Alloantigen Recognition by Clone BC4*

| Antibody | $[^3]$H]thymidine incorporation in response to:* | B10 + medium | B10 + cow insulin | PL/J |
|----------|-----------------------------------------------|---------------|-----------------|------|
| None | 181 ± 65 | 36,708 ± 952 | 30,037 ± 1,567 |
| Anti-I-A<sup>a</sup> | 1:50 | 192 ± 53 | 30,919 ± 3,290 | 31,809 ± 3,091 |
| | 1:500 | 420 ± 27 | 34,070 ± 931 | 29,789 ± 65 |
| | 1:5000 | 147 ± 37 | 27,386 ± 1,366 | 34,553 ± 861 |
| Anti-I-E<sup>a</sup> | 1:50 | 164 ± 42 | 33,416 ± 1,478 | 6,764 ± 299 |
| | 1:500 | 262 ± 44 | 34,529 ± 2,565 | 8,804 ± 1,597 |
| | 1:5000 | 300 ± 11 | 34,242 ± 789 | 14,630 ± 1,538 |
| Anti-I-A<sup>b</sup> | 1:20 | 184 ± 75 | 12,625 ± 1,629 | 30,877 ± 457 |
| Anti-I-E<sup>b</sup> | 1:50 | 245 ± 16 | NT | 34,335 ± 1,563 |
| | 1:500 | 185 ± 27 | NT | 27,096 ± 1,594 |

* $10^4$ T cells were incubated with $5 \times 10^6$ irradiated (2,000 rad) spleen cells from B10 or PL/J strains for 40 h.

† Antibody was added at the indicated final concentrations at initiation of cultures.

¶ 100 μg/ml.

¶ Monoclonal antibody 10-3.6.

¶ Monoclonal antibody 14-4-4S.

** Monoclonal antibody 25-9-17.

*** Monoclonal antibody 17-3-3S.

**TABLE VII**

*Role of Specificity Ia.7 (I-E) in Allorecognition*

| T cell clone* | Antibody | $[^3]$H]thymidine incorporation in response to spleen cells of: |
|---------------|----------|-----------------------------------------------------|----------------|------|
| | | PL/J | SM/J |
| BC4 | None | 43,214 ± 2,983 | 3,089 ± 238 |
| | Anti-I-A<sup>a</sup> | 45,140 ± 1,707 | 4,883 ± 1,114 |
| | Anti-I-E<sup>a</sup> | 5,213 ± 1,454 | 600 ± 39 |
| BC6 | None | 11,227 ± 1,419 | 2,413 ± 77 |
| | Anti-I-A<sup>a</sup> | 12,738 ± 1,496 | 5,279 ± 523 |
| | Anti-I-E<sup>a</sup> | 2,991 ± 140 | 498 ± 9 |
| BC8 | None | 83,468 ± 612 | 7,000 ± 1,036 |
| | Anti-I-A<sup>a</sup> | 85,568 ± 7,389 | 9,184 ± 1,292 |
| | Anti-I-E<sup>a</sup> | 16,107 ± 2,486 | 527 ± 82 |

* $10^4$ T cells were incubated with $5 \times 10^6$ irradiated spleen cells. Background proliferation on B10 spleen cells was: clone BC4, 851 ± 199; clone BC6, 558 ± 187; clone BC8, 439 ± 86.

† The indicated antibodies were added at initiation of cultures at a final concentration of 1:500.

† Monoclonal antibody 10-3.6.

† Monoclonal antibody 14-4-4S, recognizing specificity Ia.7.
alloreactive clones, BC4, was subcloned by limiting dilution at 0.3 cells per well. Two groups of subclones were raised. The first group was subcloned with B10-irradiated spleen cells and cow insulin; the second group was subcloned with irradiated PL/J cells. All (9/9 are shown here; a total of 16 were tested) display corecognition and allorecognition specificities identical to the parent clone (Table IV).

To further define the stimulating determinant on PL/J cells, the B10 congenic of PL/J, B10.PL, was tested. This strain has MHC genes derived from PL/J, but all non-MHC genes derived from C57BL/10. Cells from this strain were ineffective at stimulating PL/J reactive clones (Table V).

To test for the involvement of MHC gene products in this allogeneic response, monoclonal antibodies to the two class II MHC molecules of PL/J, i.e., to I-Au and I-Eu (13), were tested for their effects on PL/J recognition (Tables VI and VII). Anti-I-Au has no effect on the response to PL/J. Antibody 14.4.4S, which reacts with the Ia.7 specificity of the I-Eu molecule, inhibits recognition of PL/J cells by alloreactive anti-insulin clones; responses of the clones to syngeneic B10 spleen cells plus cow insulin are unaffected by this antibody. Conversely, anti-I-Ab antibody inhibits responses to syngeneic spleen cells and cow insulin but has no effect on the response to PL/J cells. Finally, an anti-I-E that sees a private specificity of I-Eu but is unreactive with I-Eu has no effect on alloantigen recognition (Table VI). This same fine specificity for I-Eu of PL/J mice was demonstrated for all alloreactive B10 anti-cow insulin clones. Furthermore, the small but significant response to SM/J cells (H-2v) is also inhibited by anti-Ia.7 antibody, indicating that a determinant of the I-E molecule is recognized by these clones, and the inhibition is not due to a coincidental cross-reactivity of this antibody to PL/J spleen cells (Table VII).

Discussion

It is clear from previous studies that at least some T cells express receptors both for corecognition of autologous MHC products plus antigen as well as for alloantigens (6, 8, 10, 11). Two major views have been advanced to explain the molecular and genetic basis for a cell's ability to recognize these two types of MHC molecules. The first holds that corecognition and allorecognition are mediated by distinct and independent gene products expressed on the cell (9). According to this idea, continuous stimulation of an individual's T cells by environmental antigens would not bias the repertoire for polymorphic variants of MHC products. The alternative view is that alloreactivity is due to cross-reactive recognition of alloantigens and autologous MHC molecules plus conventional antigen, and this reflects molecular mimicry between polymorphic and somatic variants of MHC products (6, 7). The latter, but not the former, hypothesis predicts that the fine specificity of a cell for conventional antigen associated with autologous MHC products will dictate its specificity for cells bearing foreign MHC product.

To distinguish between these alternatives, we compared alloreactivity patterns of T cell clones specific for identical epitopes on the insulin molecule. Although a similar study has been previously reported, the precise specificities of the antigen-specific T cell clones were not defined (11). This is critical since antigens
such as ovalbumin display many different potentially immunogenic epitopes to cells from BALB/c mice. Clones reactive to different epitopes on this glycoprotein can thus exhibit apparently random reactions toward foreign MHC products, as was found in that report. Moreover, in our hands stimulation of BALB/c cells with ovalbumin frequently generates cells that corecognize proteins in FCS associated with a foreign haplotype (Suzan Friedman, unpublished data). It is therefore critical to establish (a) a panel of clones that corecognize one epitope and self-Ia and (b) to define alloreactivity in the absence of calf serum proteins.

Analysis of a panel of clones specific for a unique amino acid sequence on the cow insulin molecule and a self-MHC product revealed that a substantial fraction of these clones (products of separate T cell stimulation experiments) reacted to one particular foreign MHC product: I-E\(^u\) from PL/J mice. All these reactions were independent of serum used to supplement the cultures. These results indicate that the gene product(s) that recognizes complexes of MHC products and conventional antigen ("Ia + X") also recognizes MHC products that mimic this complex. The similarity between complexes of I-A\(^b\) and cow insulin, on the one hand, and I-E\(^u\) from PL/J mice, is not obvious.

These results do not formally exclude the possibility that the gene product(s) that recognizes alloantigen differs from the gene product(s) that corecognizes MHC-antigen complexes. First, expression of the two gene products may be coordinately controlled in clones of inducer cells. There is no information on the sequences coding for T cell receptors, nor is there precedent for coordinate expression of two different V regions in B lymphocytes. Second, other panels of inducer T clones with defined specificity for a particular conventional antigen must be tested for selective alloreactivity to establish whether the results reported here represent a general rule rather than an adventitious cross-reactivity.

If recognition of both sets of antigens is due to a single receptor or set of receptors, the finding that 50% of B10 anti-cow insulin clones are alloreactive to PL/J and that 50% are not implies the existence of two different receptors or possibly two different gene products that recognize a very limited sequence on the cow insulin molecule. We have recently developed antibodies that bind strongly and specifically to all anti-A-loop/PL/J-reactive clones but do not bind to anti-A-loop nonalloreactive clones. These antibodies completely inhibit the responses of the anti-A-loop alloreactive clones to both cow insulin and PL/J but have no effect on the anti-cow insulin responses of the nonalloreactive clones. This reagent should define the molecular relationship of the anti-A-loop and anti-PL/J receptor(s).

Classic studies that established the high frequency of alloreactive T lymphocytes (1, 2) used noncongenic strains of rodents and thus measured alloreactivity to MHC gene products as well as complexes of these gene products with other cell surface molecules. Analogous studies using congenic mice and stringent controls for specificity of cellular activation (other than lymphokine production) are needed to quantitate the frequencies of T cells that recognize MHC gene products alone and those that recognize them associated with other cell surface molecules. The alloreaction described here may be an example of the latter type of recognition. The difference between PL/J and B10.PL with respect to activation of these clones is not apparent from these studies. One possibility is
that a non-MHC gene product of PL/J may move freely in the membrane and interact with I-E molecules such that the two determinants form one antigenic unit. Another possibility is that I-E is the sole molecule recognized by these clones. In this case, expression of the I-E gene product on B10.PL and PL/J may differ due to glycosylation differences dictated by non-MHC-encoded enzymes or because of a mutation that may have occurred during the backcrossing of this MHC haplotype onto the B10 background.

In sum, these results show that, at least for some T cells, expression of genes that dictate receptors for conventional antigen-Ia complexes and for alloantigens is not independent. They support the view that some reactions to foreign MHC products reflect molecular mimicry: foreign MHC products that successfully mimic the structure of an antigen-Ia complex recognized by an inducer cell will stimulate that T clone. Further studies of other panels of inducer clones will be necessary to determine whether the fine specificity of corecognition routinely predicts the specificity of alloreactivity.

Summary

We have analyzed a panel of T cell clones that corecognize defined epitopes of the insulin molecule in association with Ia for their patterns of recognition of alloantigens. A striking correlation is observed between recognition of the I-A\textsuperscript{b} gene product and cow insulin alpha loop and recognition of I-E\textsuperscript{u} of the PL/J haplotype. These results are consistent with the notion that reactions to foreign major histocompatibility complex (MHC) products reflect molecular mimicry by foreign class II antigens of 'physiologic' complexes formed by autologous class II MHC molecules and antigen.

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These data do not bear on the nature of the genes; i.e., possibly the germ-line genes encode cell-bound receptors for polymorphic variants of MHC products. These receptor-bearing cells are expanded by complexes of the individual's MHC and environmental antigens that mimic alloantigens.
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