

**H-2 I-REGION ENCODED TARGETS IN ALLOGRAFT
REJECTION***

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H-2 I-region encoded determinants are thought to be expressed on very few tissues including lymphocytes, macrophages, sperm, epidermal, and endothelial cells. Yet, I-region disparate allografts are rejected; not only skin (1), the epidermal cells of which are known to express Ia antigens, but other organs (2) as well. Such data have been used to argue indirectly (2) that I-region encoded determinants, which are capable of serving as targets in allograft rejection, may be present on organ parenchymal cells.

A critical examination of this question is important because in allograft systems in which donor and recipient differ for the entire major histocompatibility complex, the fact that allografts are rejected does not necessarily prove the presence of parenchymal cell targets. Evidence obtained using other transplant models has shown that the presence of allogeneic passenger leukocytes in otherwise presumably syngeneic transplants, i.e., where there are no parenchymal cell target determinants foreign to the recipient, can result in graft rejection (3-6). Thus, mere rejection of an allograft cannot be used to argue critically that there are parenchymal cell determinants that play a role in the rejection process.

The majority of fresh thyroid grafts are rejected in recipients which differ from the donor by the H-2 I region. In contrast, thyroids taken from donors pretreated with lymphocytotoxic drugs and x rays and then cultured for 10 d (cultured thyroids) are not rejected by any recipient, presumably due to depletion of passenger leukocytes. We have used transplantation of cultured thyroid lobes beneath the kidney capsule (7) to obtain direct evidence for the presence of I-region encoded parenchymal antigens which can function as targets in graft rejection. Lafferty et al. (7) have previously demonstrated that parenchymal cells of cultured thyroids carry target determinants because sensitization of the recipient with lymphoid cells syngeneic with the cultured thyroid results in rejection of that thyroid. Those experiments (which we have confirmed using cultured thyroids obtained by the protocol given in Methods), however, are most easily interpreted as indicating the presence of K/D-region encoded determinants on the parenchymal cells since donor and recipient differed by the entire H-2 complex.

The results presented here provide evidence for the existence of I-region encoded parenchymal cell antigens that can function as targets in allograft rejection because recipients presensitized to I-region different spleen cells will specifically reject cultured

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TABLE I
Fate of Fresh Thyroid Allografts Transplanted between AQR, B10.AQR, and B10.T(6R)

Fresh thyroid donor	Recipient	Ratio* in individual animals
AQR	B10.AQR	44.2, 267.1, 26.7, 317.8, 89.6, 249.8
B10.T(6R)	B10.T(6R)	80.0, 175.0, 38.5
B10.AQR	B10.T(6R)	1.1, 1.1, 20.4
B10.T(6R)	B10.AQR	1.0, 1.0, 15.8, 0.9

* Ratio of counts per minute of ^{125}I incorporated as detailed in Methods determined on day 132 after transplantation as described in Methods.

thyroids syngeneic with the sensitizing strain. These data extend our present concepts regarding the tissue distribution of I-region encoded gene products.

Materials and Methods

Preparation of Cultured Thyroids. Fresh thyroid lobes are transplanted beneath the kidney capsule (7) of recipients which have been radiologically thyroidectomized 3 wk previously with $100\ \mu\text{Ci}\ ^{131}\text{I}$. Alternatively, the donor may receive lymphocytotoxic pretreatment of 300 mg/kg cyclophosphamide on day 2 and day 1 plus 1,000-R gamma radiation before thyroid removal; the thyroid is then placed in organ culture for 10 d in an atmosphere of 95% O_2 -5% CO_2 before transplantation.¹ Cultured thyroids obtained by this protocol behave identically to long-term cultured thyroids (7, 8). That is, they show essentially indefinite survival in allogeneic recipients.

Presensitization. Thyroidectomized recipient mice are sensitized 7 d-6 mo before transplantation by intraperitoneal administration of $50\text{--}80 \times 10^6$ spleen cells.

Functional Assay. The viability of a thyroid graft is assayed as follows. The transplant recipient is given $5.0\ \mu\text{Ci}$ carrier free ^{125}I intraperitoneally. 24-48 h after injection, the recipient is anesthetized and the counts per minute emitted by the thyroid are evaluated by placing a gamma probe (components by Ortec Inc. E. G. & G., Inc., Oak Ridge, Tenn.) first over the kidney containing the thyroid and then over the contralateral control kidney. The total counts are expressed as a ratio of counts in the kidney containing the graft to those of the control kidney. A ratio which approaches one indicates graft nonfunction. The viability of the graft can thus be determined from the day of transplantation until termination of the experiment. Alternatively, 24 h after intraperitoneal administration of ^{125}I , the kidneys are placed in tubes of 10% buffered formalin for counting in a conventional gamma counter and for histological preparation. Excellent correlations exist among data obtained with the gamma probe, the conventional gamma well counter, and histological appearance of thyroids with ratios >4.0 correlating with normal thyroid morphology. Ratios given represent the averages of multiple animals tested per group.

Mice. Mice used in these experiments were bred in our own colony except that B10.AQR mice were a gift from Dr. James Finke (Cleveland, Ohio). H-2 genotypes for K, I-A, I-B, I-J, I-E, I-C, S, and D are: AQR(B10.AQR) qkkkkddd; B10.T(6R) qqqqqqd; B10.A kkkkkddd; B10.BR kkkkkkkk; B10.A(4R) kkbbbbbb; B10.A(2R) kkkkkddb; C57BL/10 bbbbbbb; A.TH ssssssd; and A.TL skkkkkkd.

Results

Fresh thyroids transplanted in the AQR-B10.T(6R) or B10.AQR-B10.T(6R) combinations are usually rejected, although not all thyroids are rejected reciprocally. Shown in Table I are results from one of seven different experiments, all yielding similar data, showing rejection in the great majority of these allografts, however, with some long-term surviving thyroid lobes. Variable results after skin allografting in this

¹ Sollinger, H. W., and F. H. Bach. Unpublished data.

TABLE II
Specific Rejection of I-Region Disparate Cultured Thyroids in Recipients Presensitized 7 d before Transplantation

Line	Cultured thyroid donor	Recipient	Donor of sensitizing cells	Ratio*
<i>a</i>	AQR	B10.T(6R)	AQR	1.0
<i>b</i>	AQR	B10.T(6R)	B10.T(6R)	9.1
<i>c</i>	B10.T(6R)	B10.T(6R)	B10.T(6R)	11.8
<i>d</i>	B10.T(6R)	B10.T(6R)	AQR	16.6
<i>e</i>	B10.BR	B10.T(6R)	AQR	1.3
<i>f</i>	B10.BR	B10.T(6R)	B10.T(6R)	14.1
<i>g</i>	B10.T(6R)	AQR	B10.T(6R)	1.0
<i>h</i>	B10.T(6R)	AQR	—	56.4
<i>i</i>	C57BL/10	AQR	B10.T(6R)	48.3
<i>j</i>	A.TH	A.TL	A.TL	249.5
<i>k</i>	A.TH	A.TL	A.TH	1.0
<i>l</i>	B10.BR	A.TL	A.TH	106.2

* Ratio of counts per minute of ^{125}I incorporated as detailed in Methods. Assays for data in lines *a* through *f* performed at 4 wk and lines *g* through *i* 7 wk after transplantation to final recipient. Results obtained in presensitized recipients are similar at any time of assay 3 wk after transplantation, i.e., rejection takes place before 3 wk and no instances of recovery have been observed as late as 15 wk.

genetic combination have been previously reported (1). In addition, in some cases, transplanted fresh thyroids show periods of nonfunction for more than 4 wk followed by recovery and excellent function. Thyroids transplanted in the A.TH-A.TL combination behave similarly.

The cultured thyroid can be used as one model to ask, *in vivo*, whether parenchymal cells carry I-region target determinants. A cultured thyroid is not rejected even in H-2 disparate recipients, presumably because passenger leukocytes are no longer present. Shown in Table II are results of experiments in which cultured thyroids transplanted into recipients presensitized 7 d earlier to spleen cells syngeneic with the thyroid donor are rejected, suggesting the presence of I-region encoded targets on thyroid parenchymal cells. An AQR cultured thyroid transplanted to a 6R recipient presensitized to AQR cells is rejected (line *a*) and vice versa (line *g*). In contrast, AQR or 6R cultured thyroids transplanted to control 6R recipients receiving 6R cells (lines *b*, and *c*) or 6R cultured thyroids transplanted to 6R recipients sensitized with AQR cells (line *d*) show highly significant function. Likewise, 6R cultured thyroids transplanted to AQR recipients that have not been presensitized (line *h*) show excellent function. Similar results were obtained when recipients were presensitized 6 wk before transplantation (data not shown).

In some experiments, the AQR strain used was not fully congenic with the background of the 6R strain. Thus, additional combinations were tested to ask whether rejection of the cultured thyroid grafts in the presensitized recipients was, in fact, due to H-2 I-region encoded determinants. Cultured thyroids from donors of the B10.BR strain, which shares the H-2 I-A, I-B, I-J, and I-E subregions with AQR and is congenic with 6R were rejected by 6R recipients presensitized to AQR cells (line *e*). Cultured B10.BR thyroids transplanted to control 6R recipients presensitized to 6R cells were not rejected (line *f*). Since the B10.BR thyroid shares the non-H-2 background genes with the 6R recipient and presents essentially the same I region to 6R as AQR, rejection is most likely related to the I-region determinants. In order to

TABLE III
*Acceptance of I-Region Disparate Cultured Thyroids in the B10.A(2R)-
 B10.A(4R) Combination Despite Presensitization*

Cultured thyroid donor	Recipient	Donor of sensitizing cells	Ratio*
2R	4R	4R	29.0
2R	4R	2R	19.9
4R	4R	2R	22.8
4R	4R	4R	79.2

* Ratio of counts per minute of ^{126}I incorporated as detailed in Methods determined on day 30.

further help rule out non-H-2-loci encoded target determinants in rejection by AQR of I-region disparate 6R cultured thyroids, C57BL/10 cultured thyroids were transplanted to AQR recipients that had been presensitized with 6R cells. If the rejection of 6R cultured thyroids by AQR recipients presensitized to 6R cells were due to non-H-2 encoded antigens, then AQR recipients presensitized to 6R cells should be sensitized to those differences and reject the cultured C57BL/10 thyroids, which does not occur (line *i*).

Similar findings, demonstrating the presence of I-region encoded target determinants on thyroid parenchymal cells have been obtained in the A.TH-A.TL combination. A.TL animals presensitized to A.TL cells do not reject a cultured A.TH thyroid (line *j*), whereas A.TL recipients presensitized to A.TH do reject the cultured A.TH thyroid (line *k*). As a specificity control, A.TL presensitized to A.TH does not reject a cultured, K-region disparate, B10.BR thyroid (line *l*).

In seven different experiments testing survival of cultured thyroid grafts in these I-region disparate combinations, 40 out of 42 cultured thyroid lobes transplanted to allogeneic recipients presensitized to leukocytes syngeneic with the thyroid donor (as in lines *a*, *g*, and *k* of Table II) were rejected (all ratios <2.5); in contrast, only 2 of the 23 cultured thyroid lobes transplanted to allogeneic recipients not presensitized, or "presensitized" with leukocytes syngeneic with the recipient, (as in lines *b*, *h*, and *j* of Table II) failed to show function.

In contrast to findings with the AQR-6R combination discussed above are those obtained in similar experiments using the B10.A(2R) and B10.A(4R) strains which differ in the I-B, I-J, I-E, I-C, and S regions, but not in I-A. These strains do not reject each other's thyroids and reciprocal sensitization does not lead to graft rejection or delays in the onset of function (see Table III).

Discussion

The findings that (*a*) both 6R and AQR animals presensitized to AQR and 6R cells respectively reject cultured thyroids of the sensitizing genotype, (*b*) that 6R animals presensitized to AQR cells reject cultured B10.BR thyroids, and (*c*) AQR recipients sensitized to 6R do not reject C57BL/10 cultured thyroids (which share the background with 6R) suggest strongly that thyroid parenchymal cells have I-region encoded target antigens. This finding is supported by the studies using the A.TL-A.TH combination. Furthermore, the finding that 4R and 2R mice do not reciprocally reject under similar conditions implies the existence of I-A-subregion encoded determinants. The formal possibility that these strains differ for loci, either close to H-2,

such as Qa, or for non-MHC genes, which are involved in these phenomena, must always be considered.

Despite the presumed presence on the parenchymal cells of determinants that can function as targets in the presensitized animal, these antigens on a cultured thyroid are apparently not immunogenic for cells that can mediate graft rejection since a cultured thyroid is not rejected. It has been suggested (2) that heart fragment rejection in I-region disparate strains argues for the presence of immunogenic, I-region encoded determinants on the heart parenchymal cells; we think it more likely that immunization by transplanted organs is dependent on the presence of passenger leukocytes or, perhaps, on some interaction between the response to passenger leukocytes and the parenchymal cell target antigens.

Because presensitized animals can recognize parenchymal cell I-region encoded targets on the thyroid lobe does not prove that these determinants have a role in graft rejection; even if they play a part in second-set graft rejection, this does not necessarily extend to first-set reactions. We have, however, obtained some preliminary data which suggest that parenchymal antigens may be important in graft destruction. When a cultured thyroid is passaged under the kidney capsule of an intermediate host for 8 d or more, and then transplanted to a final recipient whose lymphoid cells recognize K/D differences on the parenchymal cells of the thyroid and I-region antigens on passenger leukocytes acquired from the intermediate host, one observes the fibrotic sequelae of severe parenchymal cell damage which we have not seen under similar conditions when the thyroid donor and final recipient are syngeneic. Whether this, too, will apply to I-region parenchymal targets is presently under investigation.

Summary

Fresh thyroids transplanted to I-region disparate recipients are, in most cases, rejected; in some instances fresh thyroids undergo periods of crisis followed by functional recovery. Cultured thyroids that are taken from donor animals pretreated with lymphocytotoxic drugs, gamma radiation and cultured for 10 d in vitro are not rejected by any normal allogeneic recipient. If the recipient is sensitized with lymphoid cells syngeneic with an I-region disparate cultured thyroid donor, the cultured thyroid is rejected if I-A-subregion differences are included. We interpret these data to indicate that there exist I-region encoded parenchymal cell target determinants which are not, by themselves, immunogenic.

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