Alternative Self or Nonself Recognition of an Antigen Expressed in a Rare Cell Type in Transgenic Mice: Implications for Self-tolerance and Autoimmunity

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During its development, the immune system acquires the ability to recognize and respond to a wide variety of cells and other entities from the outside environment (Hood et al. 1984; Roitt et al. 1985). A necessary feature of this capacity is the ability to discriminate between what is a normal component of the body (self) and what is not (foreign or nonself). The existence of immunological tolerance toward self has been well-established for many years, and mechanisms that could serve to achieve it have been postulated and increasingly refined as our knowledge of the development, organization, and function of the immune system has progressed (Burnet 1959; Dresser and Mitchison 1968; Bretscher and Cohn 1970; Weigle 1973; Howard and Mitchison 1975; Nossal 1983; Schwartz 1989). The importance of self-tolerance can be seen in the damages wrought by autoimmune diseases, such as insulin-dependent diabetes, in which the immune system becomes self-reactive against specific cells and seeks to destroy them. In the case of diabetes, the autoimmune response succeeds in destroying the pancreatic β cells, resulting in a condition of insulin insufficiency and consequent deleterious effects throughout the body (Rossini et al. 1985). Regarding the principles of self, the possibility is evident that failures to establish or maintain a condition of nonresponsiveness toward self-antigens could be a factor in the induction of autoimmune responses. Thus, one may in principle be able to relate the conditions of tolerance and autoimmunity through the mechanisms of self/nonself recognition.

Several new approaches have contributed to our current understanding of the cellular mechanisms of self-tolerance. One of these applies the knowledge of T-cell receptor (TCR) gene structure and germ-line diversity to the analysis of the consequences of exposure to certain "super antigens" that have been found to have widespread effects on the immune response (Kappler et al. 1988, 1989; MacDonald et al. 1988; Pullen et al. 1988; Robertson 1988; Janeway et al. 1989; Mollick et al. 1989). The combination of reagents specific for particular TCR chains and an antigen that can be selectively presented has allowed the response of developing T cells to that antigen to be visualized. This experimental approach has revealed that both cell death (clonal deletion) and functional inactivation (clonal anergy or paralysis) are mechanisms for achieving selective nonresponsiveness in the T-cell compartment (Kappler et al. 1987, 1988; MacDonald et al. 1988; Rammensee et al. 1989).

A second new method for studying self-tolerance has used the stable introduction of genes into lines of transgenic mice to address different aspects of the development and maintenance of selective recognition and nonresponsiveness toward components of self. Two different and complementary strategies have proved informative about the properties of self-recognition. One involves the introduction of rearranged immunoglobulin (Ig) or TCR genes to produce transgenic mice that express the antigen receptor encoded by the transgene on a large fraction of B or T cells. This approach provides a means to visualize the response of developing lymphocytes toward self-antigens recognized by these antigen receptors and has been used to demonstrate unambiguously that both clonal deletion and clonal anergy (or paralysis) of self-reactive lymphocytes are mechanisms for achieving nonresponsiveness by B cells and T cells (Goodnow et al. 1988 and this volume; Kisielow et al. 1988a,b; Sha et al. 1988a,b; Teh et al. 1988; Nemazee and Burki 1989).

Another transgenic strategy centers on the introduction of genes that encode new self-antigens into lines of transgenic mice to evaluate the responses of the immune system to a protein that was heretofore not a normal component of a mouse but now has become a stable part of the genome and the milieu of antigens it elaborates. One of the first examples of this approach involved targeted expression to pancreatic β cells in transgenic mice of a protein called large T antigen (Tag), which is encoded within the early region of SV40 (Adams et al. 1987). Mice in one lineage were found to be selectively nonresponsive to this new self-antigen, demonstrating that tolerance to an antigen expressed on a rare cell type could be established. However, tolerance was not obligatory since mice in several other lineages were consistently not tolerant to T antigen. Moreover, mice in the nontolerant lines

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developed spontaneous autoimmunity against the transgenic self-antigen and the cells that expressed it. These two alternative responses were genetically stable within independent transgenic lines. The reproducibility of the tolerant and nontolerant phenotypes has provided a format to study the principles underlying the development of an ability to recognize and become nonresponsive toward rare self-antigens. Furthermore, it has allowed investigators to address the mechanisms by which an autoimmune response can be generated when recognition of a self-protein occurs but nonresponsiveness toward it does not. In this paper, we intend to review the characteristics of the transgenic lines that differentially express the same protein in their pancreatic β cells and then to consider the alternative immune responses to these distinct patterns of self-antigen expression.

MATERIALS AND METHODS

Hybrid genes and transgenic lineages. The transgenic lines used in this paper have been generated and propagated using standard techniques, which have been described previously (Hogan et al. 1986). The construction of the rat insulin promoter (RIP1)-Tag and rat insulin reverse (RIR)-Tag hybrid genes as well as the generation and initial characterization of the RIP1-Tag2, -Tag3, and -Tag4 lineages were presented previously (Hanahan 1985). The RIR-Tag2 line was characterized by Efrat and Hanahan (1987). The RIR3-Tag gene represents a deletion of part of the RIR-Tag regulatory region so that sequences from +180 to −278 of the insulin gene are retained, whereas those from −278 to −540 are removed. This construct and the characterization of the transgenic lineage carrying it will be described in depth elsewhere (S. Efrat and D. Hanahan, unpubl.). Unless otherwise noted, transgenic males were bred to C57BL/6J females, and transgenic progeny were identified by DNA analysis of tail biopsies.

Expression of T antigen. The immunochemical identification of T antigen expression in the various lineages was performed on tissue sections, as has been described previously (Hanahan 1985; Efrat et al. 1987; Efrat and Hanahan 1987; Alpert et al. 1988; Teitelman et al. 1988). Confirmation of T-antigen immunoreactivity as a reflection of synthesis of the bona fide protein involved immunostaining for the cellular protein p53, which can only be detected in β cells if it is stabilized by large T protein (Efrat et al. 1987). Solid tumor formation under the influence of the hybrid insulin/T antigen genes occurs in every individual, except for the RIR3-Tag2 line in which the mice only develop sporadic islet hyperplasia at old ages. The solid tumors derive from the pancreatic β cells, and all of the tumor cells express T antigen, p53, and insulin (Hanahan 1985; Efrat et al. 1987; Efrat and Hanahan 1987). The population of islets expressing the large T oncoprotein undergo various changes prior to the formation of a few solid tumors, as has been described previously (Teitelman et al. 1988; Folkman et al. 1989). The characteristics of these and other transgenic tumorigenesis models have been reviewed and discussed previously (Hanahan 1988).

Characterization of cellular and humoral responses. Cellular infiltration of the islets was analyzed first by staining of pancreatic sections with hematoxylin and eosin (H&E) and subsequently by immunostaining with antibodies for cell-surface markers that identify specific leukocyte subsets. The monoclonal antibodies used for this characterization recognize the Lyt-2, L3T4, B220, and Mac-1 cell-surface determinants on $CD8^+$ T cells, $CD4^+$ T cells, B cells, and macrophages, respectively. Their specificities and utilization have been described previously (Ledbetter and Herzenberg 1979; Springer et al. 1979; Springer 1981; Morse et al. 1982; Dialynas et al. 1983; Sanchez-Madrid et al. 1983).

Large T protein was purified from HeLa cells infected with an adeno/SV40 hybrid virus using an immunoaffinity column (Simanis and Lane 1985) prepared with the anti-T-antigen monoclonal antibody PAb419 (Harlow et al. 1981). The purified T antigen was used in the immunization experiments to assess tolerance and to serve as the solid-phase substrate in the radioimmunoassay (RIA). For immunizations, 5–10 μg of purified T protein was injected intraperitoneally into each mouse in a mixture with either complete Freund's adjuvant (CFA) (primary immunization) or incomplete Freund's adjuvant (IFA) (secondary boost).

The solid-phase RIA used for quantification of both the induced and spontaneous antibody response against T antigen is described in depth elsewhere (J. Skowronski et al., in prep.). The initial assays for both induced and autoimmune responsiveness toward T antigen, which also represent one confirmation of the specificity of the RIA, involved immunoprecipitation from extracts of radiolabeled COS cells (Gluzman 1981) that express large T antigen, using serum collected from primary or secondary immunizations of control or transgenic mice (Adams et al. 1987). For the RIA, the *Escherichia coli* protein, β-galactosidase (β-Gal), was used as an internal standard in the immunizations. A monoclonal antibody specific for T-antigen and normal mouse serum antibodies obtained by immunization with β-Gal protein were used as positive controls in the assay, and neither these control antibodies nor antisera induced in the transgenic mice against either β-Gal or T antigen cross-reacted with the other protein (J. Skowronski et al., in prep.).

RESULTS

Two Expression Patterns of Hybrid Insulin/T Antigen Genes

The basic strategy of our experimental approach has been to construct hybrid genes to direct the synthesis of

selected proteins to the pancreatic β cells in transgenic mice and then to examine both the patterns of expression and the consequences of that expression. The prototype chosen was the SV40 large T antigen. This 96-kD protein has a number of interesting properties among which are its antigenicity and oncogenic activities (Tooze 1981). Immunization with purified large T protein produces both humoral and cytotoxic T-cell responses (Chang et al. 1979; Tevethia et al. 1980). Large T is predominantly localized to the nucleus by virtue of a nuclear localization signal sequence contained within it (Kalderon et al. 1984; Lanford and Butel 1984). However, it is well established that large T can be recognized on the cell surface of many cultured cells expressing the SV40 early region. The most consistent means of identifying the cell-surface component of T antigen has been with cytotoxic T-cell lines induced in mice by SV40 viral infection or after transplantation of SV40-transformed cell lines (Trinchieri et al. 1976; Gooding 1979; Knowles et al. 1979; Gooding and O'Connell 1983; Pan and Knowles 1983; Gooding et al. 1984; Tevethia and Tevethia 1984; Pan et al. 1987; Rawle et al. 1988). These T-cell clones are class I major-histocompatibility-complex (MHC)-restricted, and given the (self)-antigen-presenting properties of class I molecules it would seem reasonable to conclude that the cell-surface component of large T is in fact processed peptides of the protein that are effectively presented in the context of class I MHC molecules. However, in certain cell lines a cell-surface component of large T antigen has also been identified by immunostaining (Deppert et al. 1980; Soule et al. 1980; Ismail et al. 1981; Deppert and Walter 1982), most recently with monoclonal antibodies specific for either amino- or carboxy-terminal epitopes of T antigen (Ball et al. 1984; Gooding et al. 1984). This raises the possibility that larger fragments of the protein are presented on the cell surface by other means. In fact, one study reported that the membrane fraction of an SV40-transformed cell line contained a 96-kD protein indistinguishable from the one present in the nuclear fraction as assessed by immunoprecipitation (Soule and Butel 1979). However, the localization of intact large T protein to the membrane has not been reported for other cell types. Nevertheless, what is clear from all of these studies is that expression of large T in a variety of cell types can be detected in living cells by components of the immune system, which indicates that the protein is effectively presented for immunological recognition on the cell surface. This quality renders the large T protein an attractive molecule for studying interactions of a new self-antigen with the immune system in transgenic mice, given that normal mice do not normally experience it.

Several different configurations of the rat insulin II gene regulatory region have been used to direct the expression of large T antigen to the insulin-producing β cells of transgenic mice. One construct aligned the insulin promoter to transcribe the SV40 early region much as it would the insulin gene (Hanahan 1985). This hybrid gene, called RIP1-Tag, has 695 bp of 5′-flanking DNA to the insulin gene, including the transcriptional enhancer, which is known to be cell-type-specific in vitro (Walker et al. 1983). In the second construct, the insulin promoter was inverted so that sequences upstream of the enhancer abutted the T-antigen-coding region, and the insulin promoter was at the distal (5′) end of the construct aligned to transcribe in the opposite direction to the T-antigen-coding region (Hanahan 1985). Surprisingly, this construct, called RIR-Tag, is expressed in a manner comparable with the RIP1-Tag hybrid gene, which utilizes the insulin promoter element. The RIR-Tag construct appears to function by virtue of a cryptic promoter element residing at the 5′ boundary of the enhancer on the opposite strand and with opposite orientation to that of the bona fide insulin promoter (Efrat and Hanahan 1987).

Both the RIP1-Tag and the RIR-Tag hybrid genes were established in several independent lines of transgenic mice. In every case, large T antigen was synthesized in the pancreatic β cells of adult mice. Given that the β cells are localized into approximately 400 focal clusters called the islets of Langerhans, which comprise only 1% of the pancreas, immunohistochemical techniques have been the primary means of identifying the cells synthesizing large T antigen. Several criteria have been used to establish the presence of large T. The most direct criterion involved the visualization of large T protein in situ with either rabbit polyclonal antisera raised against the purified protein or with one of a series of monoclonal antibodies specific for different epitopes of the protein (Harlow et al. 1981).

The presence of authentic large T protein has been confirmed by two of its properties. One is that large T binds to and stabilizes the steady-state levels of a cellular protein known as p53, so that it can be visualized by immunostaining, which is normally not possible. Thus, the presence of p53 in cells supports the conclusion that large T itself is present in an intact conformation. The second property of large T that can be used to assess transgene expression is its ability to transform cells. In most lineages, every mouse inheriting a hybrid insulin/T antigen gene inevitably developed a few pancreatic β-cell tumors and succumbed as a consequence. Biochemical analysis demonstrated that the tumors express both large T protein and p53 (Efrat et al. 1987). This supports the assumptions of the immunohistochemical analyses discussed above. An exception to the β-cell specificity has been observed in one line, RIP1-Tag2, in which about 5–10% of the mice also develop neuroendocrine tumors of the intestine capable of transcribing (S. Grant et al., in prep.). In every other case, the immunohistochemical and phenotypic evidence supports the conclusion that the insulin gene regulatory region targets expression of large T antigen to the insulin-producing β cells in adult transgenic mice.

Although mice in every line of insulin/T antigen transgenics express large T in their β cells and most develop β-cell tumors later in life, there are significant differences in the temporal patterns of expression

among the independent lines. Two general classes are evident as illustrated in Figure 1. In the first class, the transgenic mice begin to express T antigen during embryogenesis and express T antigen uniformly in their β cells as adults. Two lineages showing this pattern of developmental expression of the insulin/T antigen hybrid gene have been extensively characterized: RIP1-Tag2 and RIR-Tag2. Mice in each line first express detectable levels of T antigen beginning at embryonic day 10, both in the pancreatic diverticulum off the gut and transiently in neuroblasts in the neural tube and neural crest (Alpert et al. 1988). When insulin immunoreactivity is first evident 2 days later in the nascent pancreas, all the insulin-producing cells also coexpress large T. The transgene has a selectively wider pattern of developmental expression than the endogenous insulin genes in that it is also transiently detected in cells that express the other three islet cell hormones, as well as in neuroblasts. T-antigen expression becomes restricted to the β cells of adult mice in these two lineages, initially without consequence. However, beginning at about 6 weeks of age, abnormal cell proliferation and islet hyperplasia become evident. A period of islet hyperplasia is followed by the emergence of a few β-cell tumors that kill the mice by overproducing insulin and thereby inducing acute hypoglycemia. The characteristics of these two lineages are summarized in Table 1, and an analysis of T-antigen expression in the embryonic pancreas is presented in Figure 2.

A second and clearly distinct class of transgenic mice was also produced. Mice in these lines did not express the insulin/T antigen genes during embryogenesis nor as neonates. Rather, there was a delayed onset of transgene expression to adulthood, as is documented in Figure 1 by immunostaining for both T antigen and p53. Beginning at about 10–12 weeks, scattered β cells expressing T antigen could be detected in the islets (Adams et al. 1987; Efrat et al. 1987). Over time, the number of T-antigen-positive β cells increases until a majority (but not all) evidence transgene expression. The β cells were the only site of synthesis that could be detected, and again expression of the large T oncoprotein eventually elicited β-cell proliferation, islet hyperplasia, and the formation of solid tumors in the pancreas. The characteristics of two delayed onset lines are summarized in Table 1 for the purposes of this discussion.

The delayed onset phenotype has been observed in about one-half of the lines of insulin/T antigen transgenic mice and is stable upon continuous backcrossing to inbred mice. We attribute this pattern of expression to the chromosomal position into which the insulin gene regulatory region has become integrated. Regardless of the molecular mechanism underlying this

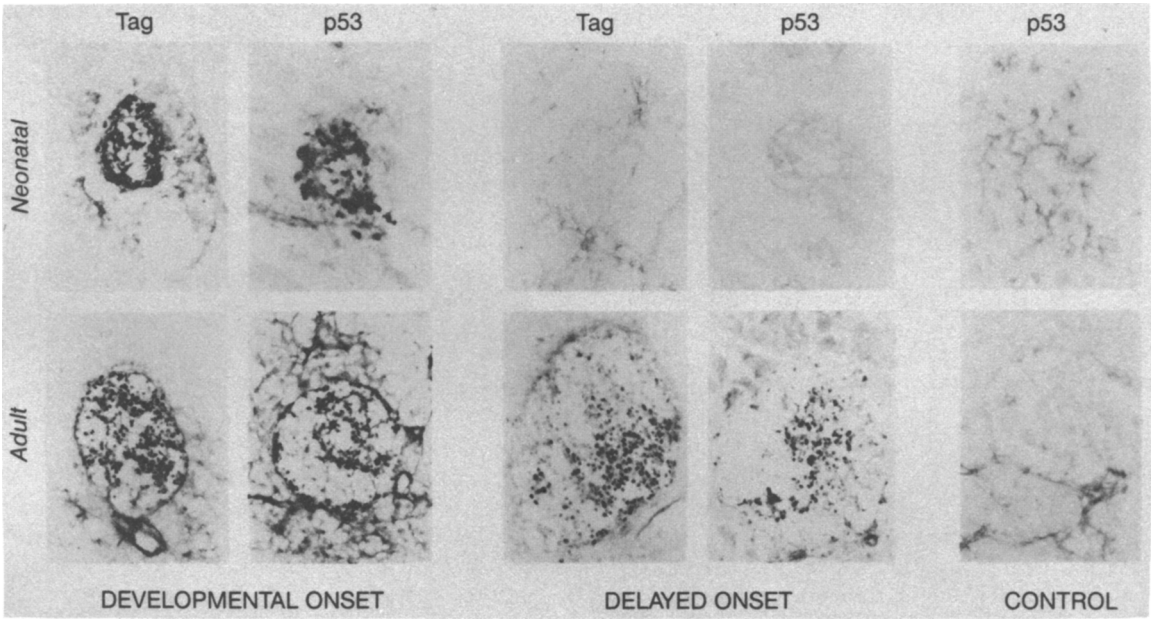

Figure 1. Two heritable patterns of transgene expression. Neonatal and young adult mice from two transgenic lineages, RIP1-Tag2 and RIP1-Tag4, were analyzed for expression of the product of the hybrid insulin/T antigen gene. T-antigen protein was detected by immunostaining pancreatic tissue sections with rabbit polyclonal antisera raised against immunoaffinity-purified SV40 large T protein, and the association was visualized by a horseradish-peroxidase (HRP)-conjugated secondary antibody specific for rabbit IgG following its reaction with diaminobenzidine (DAB), nickel sulfate, and hydrogen peroxide. Expression of authentic large T protein was confirmed by immunostaining for the mouse p53 protein, which cannot be detected in neonatal or adult controls islets (*right*). The binding of large T stabilizes p53 and renders it visible by immunostaining as is shown in neonatal islets of the RIP1-Tag2 mice and in adult islets (shown) and tumors (not shown) of both RIP1-Tag2 and RIP1-Tag4 mice. Similar patterns characterize RIR-Tag2 mice and RIP1-Tag3 mice (not shown) that thereby divide the lineages into those showing either developmental onset (*left*) or delayed onset (*middle*) (into adulthood) of expression of the insulin/T-antigen transgenes. (Data from Efrat et al. 1987.)

Table 1. Stable Tolerant and Nontolerant Phenotypes among RIP-Tag Transgenic Lineages

Lineage	Pattern of self-antigen expression	Humoral response to purified T antigen	Response to T antigen in β cells
RIP1-Tag2	developmental onset	nonresponsive	none
RIR-Tag2	developmental onset	nonresponsive	none
RIP1-Tag3	delayed onset	fully responsive	autoimmunity
RIP1-Tag4	delayed onset	fully responsive	autoimmunity

phenotype, the stability of the delayed onset phenotype has allowed us to examine the immunological consequences of this unusual temporal synthesis of a new self-antigen in the β cells and to compare them with those of transgenic mice that express the same protein during late embryogenesis and throughout postnatal life.

Stable Tolerance or Nontolerance among the RIP-Tag Lineages

One measure of tolerance toward a protein is the humoral response after immunization with that protein in a mixture with an adjuvant that stimulates the immune system. In this case, nonresponsiveness compared with controls is taken to be a condition of tolerance (Dresser and Mitchison 1968; Weigle 1973; Howard and Mitchison 1975). Self-tolerance to T antigen in the transgenic lineages was initially assessed by taking immunopurified large T protein and presenting it to both control and transgenic mice in a standard immunization regimen (Adams et al. 1987). The development of antibodies specific for large T was assessed by immunoprecipitation with extracts of radiolabeled COS cells, which is a monkey cell line expressing high levels of T antigen (Gluzman 1981). The immunoprecipitates were analyzed on SDS-protein gels. The induction of immunoprecipitating antibodies for T antigen confirmed that the protein was a potent immunogen in normal control mice. Both a primary response and an amplified secondary response could be visualized by the extent of protein immunoprecipitated, which in the secondary response was comparable with that brought down by a monoclonal antibody specific for large T. An example of this analysis is presented in Figure 3. Among transgenic mice analyzed from the different lineages, a clear pattern emerged. Mice from several lines showed a response to immunization with T antigen that was indistinguishable from the control mice, which do not carry the genetic information for the T protein. By this criterion, they were nontolerant to T antigen. In contrast, mice from another lineage (RIP1-Tag2) showed a dramatically reduced responsiveness to T antigen in that little or no large T protein was immunoprecipitated by the immunoglobulins in either primary or secondary bleeds (Fig. 3). Therefore, these mice were judged to be nonresponsive to large T and thus potentially self-tolerant to the protein whose genetic information they carry.

The alternative responsive and nonresponsive phenotypes have consistently segregated with the integrated transgenes upon their transmission to progeny as

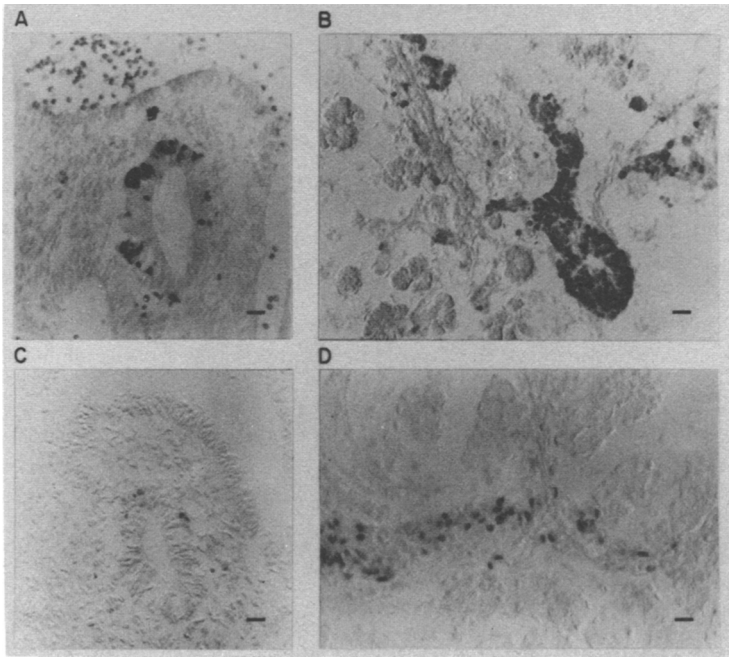

Figure 2. Differences in the levels of transgene expression during pancreatic development. Embryos from the two developmental onset lineages were analyzed for expression of T antigen. The panels illustrate consistent differences in the amount of T antigen as visualized by simultaneous immunostaining, again using an HRP/DAB histochemical reaction as described in Figure 1. (*Top*) Pancreatic sections of RIP1-Tag2 embryos at embryonic day e10 (*A*) and day e17 (*B*). (*Bottom*) RIR-Tag2 embryos at e10 (*C*) and e17 (*D*). In each case, the e10 sections are taken through the pancreatic diverticulum off the gut, and the e17 sections illustrate an islet budding away from a duct. (Reprinted, with permission, from Alpert et al. 1988.)

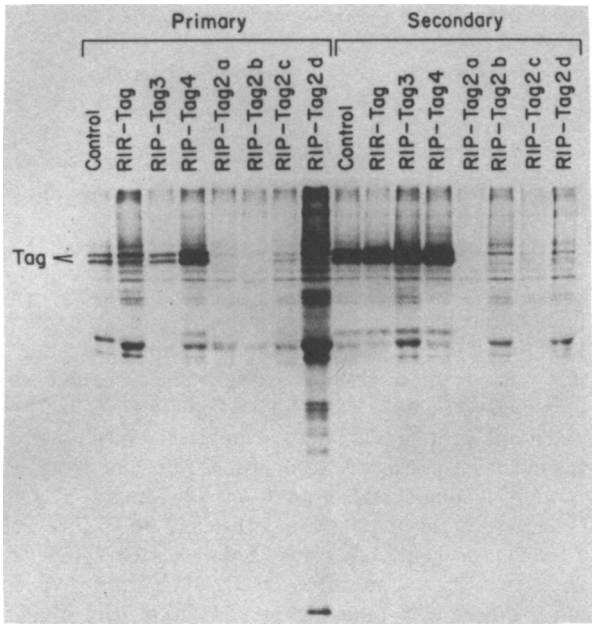

Figure 3. Two heritable types of response upon immunization with T antigen. Immunopurified large T protein was used to immunize four control mice and four mice each from several of the insulin/T antigen transgenic lineages in a standard adjuvant regimen. Both primary and secondary responses were analyzed by using serum samples to immunoprecipitate the SV40 large T antigen being synthesized in radiolabeled COS cells that express the SV40 viral genome. The immunoprecipitates were analyzed on SDS protein gels. An example is shown for one each of the control and the three delayed onset lineages, RIP-Tag3, -Tag4, and RIR-Tag1. All show a primary and an increased secondary response against large T that migrates as a dimer following immunoprecipitation from COS cells. The responses of all four mice from the developmental onset lineage RIP1-Tag2 are shown to document their nonresponsiveness relative to controls and the delayed onset transgenics. (Reprinted, with permission, from Adams et al. 1987.)

mice from a given line have all been either reproducibly tolerant or reproducibly nontolerant to T antigen using this immunization/immunoprecipitation assay. Remarkably, these two distinctive immunological characteristics correlate with the two classes of transgene expression. Mice from the RIP1-Tag2 line that expressed T antigen during embryogenesis were immunologically nonresponsive as adults, whereas mice from the RIP1-Tag3 and RIP1-Tag4 lines showing delayed onset of β-cell-specific expression of T antigen were fully responsive to the purified protein (Table 1). Thus, among a series of independent lines of transgenic mice, two genetically stable immunological phenotypes were produced toward a self-protein that was known, well-characterized, and available in purified form as were the specific immunological reagents to detect it. These characteristics, in conjunction with the knowledge of the temporal and spatial expression patterns of T-antigen expression, present an opportunity for detailed studies on the requirements for the establishment of tolerance and the qualities and the consequences of nontolerance to a self-antigen. Both issues have proved amenable to study, and the current status of our investigations into each is presented below.

Consequences of Nonself Recognition

Transgenic mice in the delayed onset class are fully responsive upon presentation of exogenous large T protein. This raises the question of whether they are responding to the same protein being synthesized in their pancreatic β cells. A screen of random serum samples revealed that a subset of the delayed onset mice had circulating autoantibodies that were capable of selectively immunoprecipitating large T antigen (Adams et al. 1987). Autoantibodies were never seen in the tolerant lines. Thus, nontolerance and delayed onset of T-antigen expression resulted in sporadic humoral autoimmunity. More extensive sampling allowed statistical analysis of the appearance of T antigen autoantibodies. Every nontolerant line had an appreciable incidence of autoantibodies, which was 65% in randomly bred and sampled RIP1-Tag3 mice and 35% among RIP1-Tag4 mice. The fact that nontolerance did not obligate autoantibodies suggested that additional factors to the delayed onset of T-antigen expression might be involved in the induction of the autoimmune reaction.

To dissect further the development of the humoral autoimmune response against T antigen, a temporal study was conducted over the lifetimes of a number of members of the RIP1-Tag3 lineage. We exploited the ability to collect serum samples and thereby noninvasively monitor the development of circulating autoantibodies against the large T protein. The study was broadened to encompass another possible factor in the variable incidences of autoimmunity, namely, genetic differences in general and specific differences in the MHC haplotype in particular. Many diseases of apparent autoimmune nature are associated with and presumably influenced by specific haplotypes of the MHC complex (Todd et al. 1987; Wraith et al. 1989). Correlations of this type could be presumed to reflect a differential ability of allelic MHC molecules to present peptides of the autoantigens that are inducing the immune response. In this regard, it is significant that the original founders for the insulin/T antigen lineages developed from F_2 embryos derived from C57BL/6J and DBA/2J inbred mice, which carry the H-2^b and H-2^d MHC complexes, respectively. Thus, each founder was a mixture of these two genotypes. The transgenic lines were initially established by backcrossing to C57BL/6J and also by intercrossing to produce homozygotes for the transgene. Each line therefore contained a mixture of two genotypes and potentially of the two MHC haplotypes, at least in the early generations, which motivated an evaluation of possible genetic influences on the incidence of autoimmunity.

A time course of autoantibody production was conducted on RIP1-Tag3 mice that were derived from a transgenic male parent that had been haplotyped as homozygous for the H-2^b MHC and then mated with

inbred females. A preliminary screen of four different backcrosses to C57BL/6, DBA/2, C3HeB, and B10.BR showed variable incidences and titers of anti-large T antibodies at 5–5.5 months of age, with the two parental backgrounds C57BL/6 and DBA/2 being the most diverse in both parameters (not shown). Therefore, backcross progeny of RIP1-Tag3 (H-2^b/H-2^b) to either C57BL/6 (H-2^b) or DBA/2 (H-2^d) were routinely examined for serum autoantibodies beginning at 3 months and continuing throughout their lives (Fig. 4). It is evident that there are genetic differences in the responses seen in the two cases. Mice homozygous for the H-2^b MHC show a low incidence and a low titer of autoantibodies. In marked contrast, mice carrying both H-2^d and H-2^b showed a 100% incidence of autoantibodies. The serum titers were substantially higher, reaching levels comparable with those induced by immunization of mice with purified large T protein. In both cases, spontaneous autoantibodies were first apparent beginning at 4 months of age, which is 4–6 weeks after the synthesis of T antigen in the β cells ensues. From this, we infer that the appearance of T antigen can result in the induction of autoantibodies and that there is a genetic component to the character of that response.

The implication from the time course of a genetic control of autoimmunity raised the prospect that the distinctive MHC alleles were a major factor, given their association with various autoimmune diseases and the demonstrated ability of both class I and II molecules to present antigens to the immune system. This possibility has begun to be addressed from several perspectives. To assess the specific contribution of MHC alleles, RIP1-Tag3 males were backcrossed to C57BL/10 mice and to B10.D2 mice. C57BL/6 and C57BL/10 are closely related, and B10.D2 is congenic with C57BL/10 except for the presence of the H-2^d MHC complex. In preliminary results from this analysis, RIP1-Tag3 backcrosses to C57BL/10 were found to be indistinguishable from C57BL/6 with regard to incidence and titer, whereas those to B10.D2 were similar to DBA/2. A second experiment outcrossed the H-2^b/H-2^b transgenics to B6D2 mice, which are F_1 hybrids of C57BL/6 and DBA/2. These progeny were analyzed both for autoantibodies and MHC haplotype, and again the incidence of autoimmunity was substantially higher in the

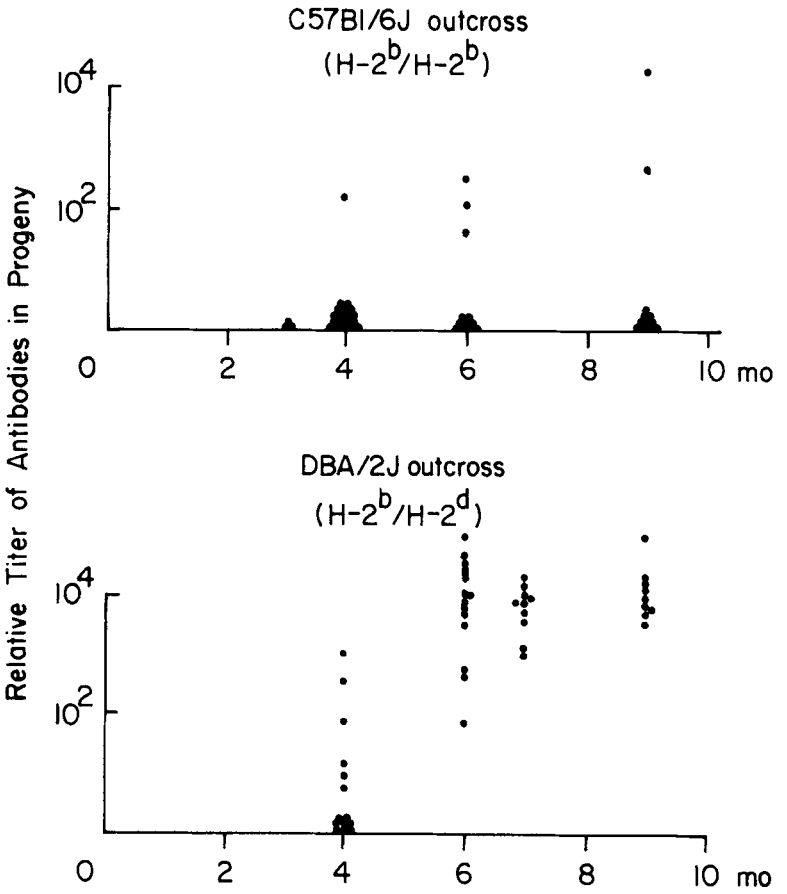

Figure 4. Time course of T-antigen autoantibodies in two MHC backgrounds. RIP1-Tag3 males (H-2^b/H-2^b) were mated with C57BL/6J (H-2^b) or DBA/2J (H-2^d) females, and their transgenic progeny followed over the course of their lives for the development of serum autoantibodies against T antigen. Each dot represents the analysis of serum taken from one individual at the timepoint indicated on the ordinate. The titer of anti-T-antigen antibodies is given on the axis as determined using a quantitative solid-phase RIA (J. Skowronski et al., in prep.). Some individuals did not live to reach the later timepoints.

mice carrying an H-2^d allele (Fig. 5). These analyses are consistent with the interpretation that the H-2^d allele confers a strong predisposition for development of humoral autoimmunity, whereas H-2^b alone is considerably less effective in inducing the response. Further support for the conclusion that allelic differences in the MHC influence the autoimmunity comes from crosses to mice carrying H-2^k, in which transgenic progeny show an intermediate incidence and intensity of the humoral response to T antigen. It should be mentioned that the transgenic mice of every H-2 type evidence similar patterns of expression of T antigen in that onset occurs beginning at 10–12 weeks, and by 20 weeks virtually every one of the pancreatic islets contains β cells synthesizing T antigen.

The cellular response toward the β cells expressing T antigen has initially been addressed by histological analyses of tissue sections. Evaluation of pancreases from mice in the RIP1-Tag3 lineage revealed lymphocyte infiltration of the pancreatic islets in virtually every mouse, as indicated by staining with H&E. A series of

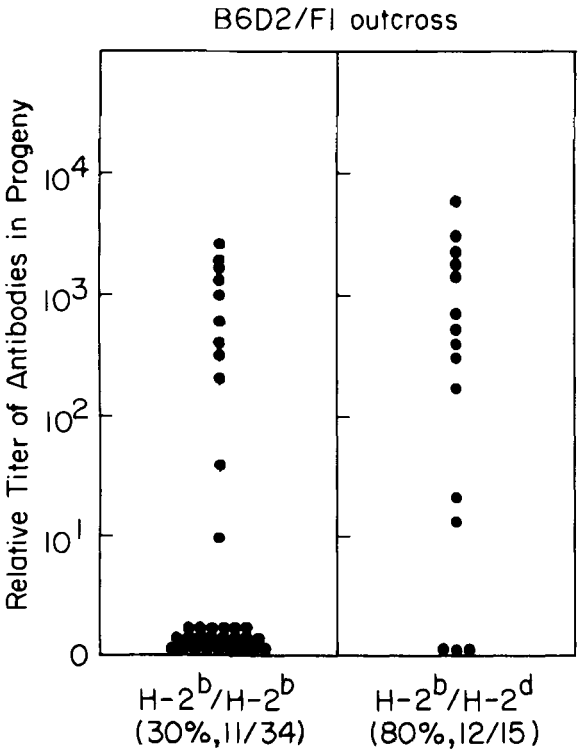

Figure 5. Relation of autoantibody response to segregating MHC haplotypes. RIP1-Tag3 males were outcrossed to B6D2F, females, and their progeny were analyzed for autoantibodies at 6 months and subsequently typed for MHC haplotype using restriction-fragment-length polymorphism analysis for the Aβ chain of the class II I-A gene as described elsewhere (J. Skowronski et al., in prep.). Note that, whereas H-2^d MHC confers the high-frequency incidence, other alleles in the DBA background appear to influence the titer of antibodies that develop in the H-2^b/H-2^b MHC background. (Compare this 6-month timepoint of H-2^b/H-2^b mice with that in Fig. 4).

monoclonal antibodies that recognize specific lymphocyte subsets was used to identify the cells infiltrating the islets as either $CD8^+$ cytotoxic T cells, $CD4^+$ T helper cells, B cells, or macrophages, by virtue of their expression of the Lyt-2, L3T4, B220, and Mac-1 determinants, respectively. Immunostaining of pancreatic sections showed that all four classes of leukocytes could be detected in the infiltrated islets. In contrast, nontransgenic control mice showed only rare macrophages associated with the periphery of the islets. The extent of infiltration varied among the islets of an affected individual, with some islets being highly infiltrated whereas others were unaffected. This heterogeneity of infiltration is similar to that observed in a number of cases of natural autoimmunity against the pancreatic β cells such as in human type-1 diabetes (Gepts and Lecompte 1988) and in the diabetes seen in the BB rat and the nonobese diabetic (NOD) mouse (Fujita et al. 1982; Kanazawa et al. 1984; Logothetopoulos et al. 1984; Dean et al. 1985). When examined at 5 months of age, every RIP1-Tag3 mouse evidenced cellular infiltration. An example of this analysis is presented in Figure 6. What is clear is that within 2 months of the delayed onset of T-antigen expression in the pancreatic β cells of RIP1-Tag3 mice, every mouse had developed leukocyte infiltration of the pancreatic islets consisting of B and T cells and macrophages. This infiltration is not evident in mice from the same line at 3 months of age when expression of T antigen is just ensuing.

Regarding the cellular response to T antigen, it is remarkable that, whereas every islet expresses T antigen by 4–5 months, in an H-2^b/H-2^b background only 30% of the mice develop autoantibodies during their lifetimes despite the fact that all eventually succumb to pancreatic β-cell tumors. This dichotomy has motivated further comparison of RIP1-Tag3 mice derived from backcrosses to low-responder (C57BL/10) and high-responder (B10.D2) MHC backgrounds. The transgenic progeny derived from these crosses were analyzed for lymphocyte infiltration initially at 5 months as part of a time course of the cellular response. In all four cases, leukocyte infiltration of the islets could be detected. There was variation among the islets of an individual regarding the extent of infiltration as was discussed above. However, there was no obvious difference between the four cases regarding the number of infiltrated islets nor the types of leukocytes that were infiltrating. The only variation was that in the autoantibody-positive, high-responder H-2^d background there appeared to be substantially more B cells within the islets. The numbers of $CD4^+$ and $CD8^+$ T cells and macrophages were similar in antibody-positive and -negative mice of either low- or high-responder MHC haplotype (not shown). These results are provocative because they imply that the immune system in all cases recognizes and is attracted to the location of T-antigen synthesis but that attraction does not necessarily lead to activation of the immune response, as measured by the appearance of circulating autoantibodies to T antigen.

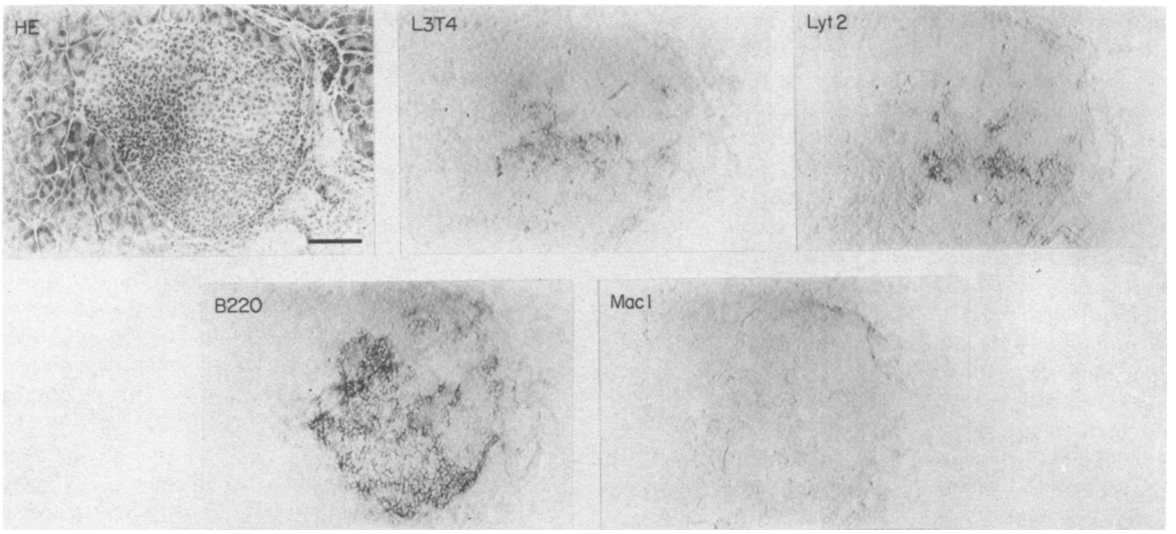

Figure 6. Leukocyte infiltration of islets in transgenic mice nontolerant for T antigen. RIP1-Tag3 (H-2^b/H-2^b) males were outcrossed to B10.D2 (H-2^d/H-2^d) females, and their transgenic progeny were analyzed for leukocyte infiltration at various timepoints. The representative example shown here is of an autoantibody-positive transgenic mouse at 5 months of age. The panels show histochemical staining with H&E and immunostaining with antibodies that recognize the following determinants: L3T4 on $CD4^+$ T-helper cells; Lyt-2 on $CD8^+$ cytotoxic T cells, B220 on B cells, and Mac-1 on macrophages. The bar represents 100 μm.

Characteristics of the Tolerance Induced by Developmental Expression

Developmental onset of expression and its continuation throughout life in the pancreatic β cells of RIP-Tag2 mice leads to a nonresponsive condition. This conclusion was initially based on assays in which the mice were immunized with purified large T in adjuvant and the response assessed by immunoprecipitation of extracts from radiolabeled COS cells with serum collected before and after primary and secondary immunizations, as was described above. These assays did not provide information about the general responsiveness of mice in the various lineages nor regarding possible differences in the extent of their nonresponsiveness. To address both of these issues, a semiquantitative solid-phase RIA was established. Equal quantities of large T protein and the *E. coli* protein β-Gal were used to immunize both control and transgenic mice in a standard regimen of CFA followed by IFA. Serum from prebleeds and bleeds taken after the primary and secondary immunizations were serially diluted and assayed for binding to either large T or β-Gal proteins immobilized on a solid phase. The binding reaction was visualized with an iodinated secondary antibody reactive with IgG and quantitated in a gamma counter. The use in parallel of antibodies specific for large T and for β-Gal allowed relative titers to be established from the half-maximum of the dilution curves. There was no cross-reactivity between T-antigen and β-Gal as assessed by their separate immunization and evaluation with the two control antibodies (J. Skowronski et al., in prep.).

Table 2. Characteristic Nonresponsiveness to Large T Antigen

	T-antigen expression				Immunological responsiveness to T antigen	
Mouse lineage	onset	relative levels[a]	stabilization of p53[a]	β-cell tumors	by immunization with Tag[b]	by lymphocyte infiltration of islets[c]
C57BL/6J	—	—	—	—	1	none
RIP1-Tag3	10–12 weeks	(−)→++	(−)→++	30–60 weeks	1	extensive
RIP1-Tag2	e10	+++	+++	10–14 weeks	$<10^{-3}$	none
RIR-Tag2	e10	++	++	16–24 weeks	10^{-1}–10^{-2}	none
RIR3-Tag2	n.d.	+	+	none (hyperplasia)	1/2	none

[a] Relative levels of T antigen were determined by immunostaining adjacent sections with antibodies to SV40 large T and mouse p53 that were visualized with HRP-conjugated secondary antibodies upon reaction with DAB. Comparisons between the lines were established following DAB reactions performed in parallel on several occasions. n.d. indicates not determined.

[b] Sera collected after immunization with purified large T protein in CFA/IFA were titered for antibodies to large T by a solid-phase RIA. The titers are given relative to C57BL/6J control mice, which develop an antibody titer of $\sim 5 \times 10^4$/ml. All mice responded with similar titers of anti-β-Gal antibodies when coimmunized with purified *E. coli* β-Gal as part of the same regimen.

[c] Leukocyte infiltration assessed by H&E staining of pancreatic tissue sections fixed with paraformaldehyde.

Groups of four individuals from control mice (B6D2) and from the transgenic lineages were analyzed for their responsiveness to T antigen and β-Gal. The results are summarized in Table 2. All of the mice responded similarly to β-Gal, which indicates that the transgenic mice are not generally immunodeficient. Comparison of the responses to T antigen confirmed the previous observation that RIP1-Tag2 mice are nonresponsive. The control nontransgenic mice mounted a discernible primary and a strong secondary response to T antigen. In contrast, RIP1-Tag2 mice did not evidence a primary response, and the second response was either undetectable or barely detectable in this assay, giving a relative titer of $<10^{-3}$ when compared with those of the controls. The analysis of mice in the RIR-Tag2 lineage also demonstrated nonresponsiveness but to a lesser extent. In these mice, a primary response was again not evident, and the secondary response was reduced to 10^{-1} to 10^{-2} of that of the control mice. The nonresponsiveness upon immunization was reproducible and judged to be significant since mice in this line are tolerant by the criteria that they do not spontaneously develop either autoantibodies against T-antigen or leukocyte infiltration of the pancreatic islets.

There is a clear correlation between onset of T-antigen expression during embryogenesis and the subsequent nonresponsiveness of the mice as adults. Thus, neither RIP1-Tag3 mice nor RIP1-Tag4 mice express T antigen during development, and each are nontolerant and fully responsive. Both RIP1-Tag2 and RIR-Tag2 mice express T antigen beginning at embryonic day 10 (Fig. 2) and continue to do so throughout their lives. The expression of large T during the primary development of the immune system therefore appears to be a necessary condition for the establishment of self-tolerance to this protein when it is expressed as an antigen in the pancreatic β cells.

A second characteristic of the establishment of self-tolerance indicated in these transgenic experiments is a relationship between the levels of expression of the self-antigen and the subsequent degree of immunological nonresponsiveness toward that antigen. Although both RIP1-Tag2 and RIR-Tag2 mice begin to express T antigen in the pancreas and the nervous system at embryonic day 10, there are consistent differences in the levels of T antigen throughout embryogenesis and subsequent postnatal life. Three criteria support the conclusion of quantitative differences in T-antigen expression: (1) Immunostaining for large T itself with both rabbit polyclonal and mouse monoclonal antibodies shows reproducible differences in the intensity of immunoreactivity; (2) immunostaining for the cellular protein p53, which can only be seen as a stable complex with large T, shows similar differences in immunoreactivity between the two lines; and (3) mice in the RIP1-Tag2 line develop tumors and die between 12 and 16 weeks of age, whereas in RIR-Tag2 mice this occurs between 16 and 24 weeks of age. Thus, we conclude that there are significant differences in the amounts of T antigen produced in the two lineages. Moreover, these differences influence the degree of nonresponsiveness that is established, giving a relative responsiveness of $<10^{-3}$ in RIP1-Tag2 mice and 10^{-1} to 10^{-2} in RIR-Tag2 (Table 2).

The evidence for quantitative effects on nonresponsiveness toward self-antigens is further supported by analysis of a third line RIR3-Tag2. Mice in this line express even lower levels of both T antigen and p53 as assessed by immunostaining of young adults (S. Efrat and D. Hanahan, unpubl.). Significantly, these mice do not develop solid tumors although β-cell hyperplasia can arise in older mice. All three criteria indicate that T antigen is expressed but at very low levels. When mice in this line were analyzed after immunization with T antigen and β-Gal, they were found to be no more than two-times less responsive than controls. Yet, these mice do not develop spontaneous lymphocyte infiltration of the islets. Thus, we suspect that the reduced humoral response upon immunization is significant. However, it is also possible that the levels of T antigen are simply too low to be effectively presented for induction of either self-tolerance or autoimmune responses to it. These observations clearly motivate us to extend the tolerance assays into the T-cell compartment.

A final aspect of self-tolerance that has been explored is the possibility that specific haplotypes of the MHC influence the self-learning process. This possibility was suggested by the effects that MHC haplotypes have on the incidence and intensity of the autoimmunity that develops in the delayed onset class of transgenic mice. One might suppose that whichever MHC molecule was presenting T-antigen peptides during the induction of autoimmunity would also be similarly effective in the establishment of self-tolerance in those circumstances where T antigen was present during immunological development. This possibility was addressed in mice from the RIR-Tag2 lineage. These mice show a reduced but measurable response, and therefore we

Table 3. Assessing MHC Influences on Tolerance in RIR-Tag2 Mice

Genetic background (F × M)	MHC haplotype of progeny	Responsiveness to T antigen[a]	Responsiveness to β-Gal[a]
C57BL/10J × C57BL/10J	H-2^b/H-2^b	1	1
C57BL/10J × RIR-Tag2	H-2^b/H-2^b	10^{-2}–10^{-3}	1
B10.D2 × RIR-Tag2	H-2^b/H-2^d	10^{-2}–10^{-3}	1
B10.Br × RIR-Tag2	H-2^b/H-2^k	10^{-2}–10^{-3}	1

[a] Responsiveness is given relative to nontransgenic control mice (C57BL/10J). In each case, 10 μg of T antigen plus 10 μg of β-Gal were used to immunize mice in a standard Freund's regimen. The titers of antibodies in the secondary response of the controls was about 5×10^4 for Tag and 1×10^5 for β-Gal.

reasoned that any changes in the degree of nonresponsiveness conferred by different MHC alleles could be detected. RIR-Tag2 mice in an H-2^b/H-2^b background were crossed to C57BL/10, B10.D2, and B10.BR mice, which carry the b, d, and k haplotypes, respectively. The transgenic progeny of these matings were immunized and analyzed for their responsiveness to T antigen. No significant differences in the extent of nonresponsiveness were detected in the three MHC backgrounds (Table 3) from which we conclude that there is no obvious effect of these alleles on the tolerance process.

DISCUSSION

The lines of transgenic mice carrying hybrid insulin/T-antigen genes constitute an attractive model with which to explore interactions of the antigens expressed in rare cell types with the immune system. The two genetically stable patterns of transgene expression have revealed a fortuitous if somewhat mysterious phenomenon. Of particular interest is the delayed onset of β-cell-specific expression into adulthood, a condition that has been observed in about one-half of the independent lines of transgenic mice carrying hybrid insulin genes. The stability of the phenotype after continuous backcrossing to various strains of inbred mice suggests that the delayed onset is dictated by chromosomal position. The question then arises as to whether this is a special feature of the insulin gene regulatory region, the pancreatic β cell, or both. In this regard, it is of note that all four of the transgenic lines produced with a glucagon promoter/T-antigen gene express T antigen during embryogenesis (Efrat et al. 1989). This result suggests that neither the T-antigen gene nor bacterial plasmid sequences are responsible for the delayed onset since both of these sequences are in common with the hybrid insulin genes. Yet, of the 11 insulin/T-antigen lines generated, 6 evidenced the delayed onset phenotype. Moreover, several other hybrid insulin genes appear to be showing either delayed onset or heterogeneous expression phenotypes (Edwards et al. 1989; and our unpublished observations). Thus, one might suspect that either the β cell and/or insulin gene regulatory elements are particularly sensitive to the chromosomal location into which a β-cell-specific gene has been integrated. We have recently studied the transgene integration sites in four of these lineages using plasmid rescue into *E. coli* strains that carry mutations in methylated cytosine-dependent restriction activities. The results clearly indicate that the delayed onset integrations are much more highly modified (methylated) than those showing developmental onset, in a manner that could be presumed to influence their accessibility to transcription (S. Grant et al., in prep.). One could speculate that normal cellular genes might also be integrated into chromosomal locations that are subject to this type of epigenetic control that might render their expression irregular in cell types such as the β cell. The delayed onset or heterogeneous expression of these genes may be functionally or immunologically significant in disease states of the β cell such as in diabetes.

Whatever the explanation for the epigenetic control of transgene expression, the different patterns of expression have proven to be extremely valuable in that we have had the opportunity to compare the immunological consequences of expressing the same antigen either during embryogenesis and subsequent life or beginning only during adulthood. Moreover, the antigen, the large T protein of SV40, is well-characterized, available in purified form, and readily detectable using either polyclonal or monoclonal antibodies. The two temporal patterns of expression produce two consistent immunological responses. Onset during embryogenesis confers nonresponsiveness toward large T protein, as assessed both by immunization with purified protein and by the lack of autoimmune phenomena. In contrast, delayed onset results in nonself recognition of T antigen as demonstrated by strong responsiveness toward exogenous protein and the frequent induction of an autoimmune response against what is a "self-protein" and the cells that synthesize it. These transgenic mice are thereby allowing investigations into the mechanisms of alternative self-tolerance and autoimmunity for a β-cell antigen.

Principles of Self Recognition

A comparison of the developmental onset and delayed onset lines of transgenic mice has revealed that the presence of the self-antigen during the primary development of the immune system is necessary to establish a condition of nonresponsiveness or self-tolerance toward T antigen expressed in the pancreatic β cells. This result supports the classical concept of a temporal window of self-education during which a developing immune system comes to recognize and become nonresponsive toward components of its host organism (Burnet 1959). Among the transgenic lines with developmental onset, different levels of T antigen are apparent by several criteria. These levels seem to correlate with the subsequent degree of nonresponsiveness as assessed by the humoral response upon immunization with purified large T protein. Yet, all the developmental onset mice are similarly tolerant by the criterion that none evidence spontaneous autoimmune responses against the endogenous protein or the β cells that synthesize it. It is clear that the T-cell response to exogenous T antigen should now be examined to ascertain the characteristics of both helper and cytotoxic T cells when presented with the protein.

The set of developmental onset lines, with their distinctive levels of expression, should prove valuable in future investigations into the cellular mechanisms underlying the nonresponsive condition. Other studies in transgenic mice and in mice with polymorphic self-antigens have revealed that both clonal deletion and functional inactivation of self-reactive lymphocytes are mechanisms by which selective nonresponsiveness is achieved (Kappler et al. 1987, 1988; Goodnow et al.

1988; Kisielow et al. 1988a,b; Sha et al. 1988a,b; Teh et al. 1988; Blackman et al. 1989; Matzinger and Guerder 1989; Nemazee and Burki 1989; Scott et al. 1989). Most of these studies have used antigens that are widely dispersed and presumably accessible to the thymus and fetal liver/bone marrow in which the primary development of the immune system takes place. However, it is important to ask what happens in the case of antigens expressed in rare cell types? It is not clear how the information about the components of rare cells is communicated to the developing immune system. One possibility is that there is a mechanism for transporting information back to the major lymphoid organs, perhaps by macrophages or T cells that are specially instructed during the self-learning period. Alternatively, there may be mechanisms for establishing and maintaining self-tolerance in the periphery. The recent experiments of Miller and his colleagues using a polymorphic H-2 antigen expressed in the β cells of transgenic mice have provided evidence for a peripheral tolerance mechanism (Morahan et al. 1989; Miller et al., this volume). It will be of interest to compare their results with MHC antigens with those obtained with non-MHC antigens such as T antigen, especially considering the likelihood that antigen presentation by the MHC is involved in the establishment of self-tolerance. In this regard, it is notable that our results to date have not revealed variations among distinct MHC alleles in the degree of nonresponsiveness that is established by developmental expression in contrast with the apparent MHC influences on the incidence and intensity of humoral autoimmunity.

One could propose that the MHC molecules involved in antigen presentation during self-learning are different from those that act in the induction of an autoimmune response. Thus, one type of MHC molecule could be involved in the establishment of self-tolerance to T antigen in the developmental onset mice, whereas a different MHC gene mediates the induction of autoimmunity in the delayed onset RIP-Tag mice. Perhaps the *attraction* of lymphocytes to the islets in the nontolerant mice involves the same MHC gene that acts in the establishment of tolerance (e.g., a class I gene), whereas the *activation* of the autoimmune response involves a distinct gene (e.g., a class II gene) that shows differential ability to present T antigen among its alleles.

A Model of Autoimmunity

The failure to express T antigen during embryogenesis has two important and interrelated consequences. The first is that the mice do not come to recognize this self-antigen as such, despite the fact that its gene is a stable component of the germ line and hence is genetically self. This result supports the proposition that self-antigen expression during immunological development is important for subsequent self-tolerance and would seem to argue against the notion that every protein coding region is somehow transiently expressed in the developing thymus so as to effect tolerance. The second consequence of delayed onset of β-cell-specific expression is autoimmunity, which is manifested at both the cellular and humoral levels. The characteristics of the autoimmune process are intriguing and have the prospect of being a model for other cases of autoimmunity in which the autoantigens are presently unknown or inaccessible.

Humoral autoimmunity is detected beginning at least 4–6 weeks after the synthesis of large T is initiated in the β cells. Both the incidence and the titer of circulating autoantibodies appear to be influenced by the MHC haplotype. Significant correlations of MHC haplotype with several autoimmune diseases have been noted previously (Todd et al. 1987; Wraith et al. 1989; McDevitt et al., this volume), and this model appears to share that property, which supports its significance and generality. It is now well-accepted that differential autoantigen presentation by MHC molecules is likely to underlie the observed correlations between autoimmunity and MHC haplotype. SV40 T antigen elicits distinguishable cytotoxic T-cell responses among mice of different MHC haplotypes that were either immunized with cells expressing large T antigen or infected with SV40 virus. However, the MHC correlation in the cytotoxic assays is opposite to that observed for the humoral response in this model. Here, we see H-2^d conferring a 100% incidence of humoral autoimmunity and a titer of autoantibodies approaching that achieved by immunization of control mice with purified large T antigen. In contrast, H-2^b confers an incomplete incidence (30%) and lower titers of autoantibodies. These associations are opposite of those observed in cytotoxic T-cell assays performed either in vitro or via tumor transplantation, where H-2^b is a high-responder and H-2^d is a low-responder haplotype (Gooding 1979; Knowles et al. 1979; Pan et al. 1987).

The dichotomy between the autoimmune response and the tumor transplantation response to T antigen suggests that the MHC genes involved in the autoimmune reaction against the β cells are different from those that participate in the response against large T as a tumor transplantation antigen. In this regard, it is of note that the autoimmunity in these mice does not confer tumor immunity. The mice eventually succumb to the effects of β-cell tumors and not insulin-dependent diabetes. The solid tumors in general appear relatively devoid of lymphocyte infiltration, in contrast with the islets that not only show significant leukocyte infiltration but also evidence of varying degrees of β cell destruction (Adams et al. 1987; C. Jolicoeur, unpubl.). Thus, it appears that the β-cell tumors manage to avoid the attentions of the immune system, perhaps by attenuating class I MHC expression or by modulating its presentation of the large T autoantigen. It has been shown previously that normal β cells express class I MHC (Baekkeskov et al. 1981; Allison et al. 1988) and the β-cell tumors continue to express class I molecules as assessed by immunoprecipitation (S. Baekkeskov, pers. comm.). Therefore, one might suggest that differ-

ences in antigen presentation are responsible for the relative invisibility of the tumors.

The mechanism of immunological recognition and response to T antigen following its delayed appearance in the β cells of an adult mouse is proving to have unexpected complexity. Contrary to our simplest expectations, lymphocyte infiltration was observed in all cases beginning 4–6 weeks after synthesis of T antigen ensued. Thus, regardless of the MHC haplotype of the RIP1-Tag3 transgenic mice, lymphocytes quickly recognized the presence of something unusual in the pancreatic islets and therefore began to infiltrate them. Four classes of leukocytes were evident in both high-responder (H-2^d/H-2^b) and low-responder (H-2^b/H-2^b) backgrounds: $CD4^+$, $CD8^+$, $B220^+$, and Mac-1^+ cells, representing helper and cytotoxic T cells, B cells, and macrophages, respectively. Yet, despite the fact that immunological recognition of T-antigen synthesis in the β cells occurs in every mouse, activation of the humoral immune response is not an obligatory consequence of infiltration. Moreover, the MHC haplotype appears to influence activation of the humoral autoimmunity, rather than recognition of the nonself-antigen per se. It is particularly notable that in low-responder H-2^b mice, lymphocytes can infiltrate and persist in the islets throughout the lives of these individuals and yet not become activated to produce antibodies. This is especially remarkable when one again recalls that these mice develop β-cell tumors as a consequence of T antigen synthesis, and even tumorigenesis does not activate humoral autoimmunity against T antigen.

It will now be of interest to compare the infiltrating leukocytes in both low- and high-responder MHC backgrounds using reagents that identify functionally activated cells to determine whether the infiltrating T cells and macrophages only become activated in certain cases, as are the B cells, and to ascertain whether that activation is influenced by particular MHC haplotypes. This model for the recognition of a nontolerant self-antigen by the immune system appears to have revealed a significant property, namely that the *attraction* of the immune system is functionally separable from its *activation*. In this regard, it will be important to subdivide the MHC complex using recombinants between H-2^d and H-2^b in order to identify the MHC gene responsible for activation of the immune response. Separation of attraction and activation has also been inferred from genetic studies of the NOD mouse. Certain genetic outcrosses evidenced lymphocyte accumulation around the islets (attraction) but not destructive autoimmunity, whereas in other backcrosses and in the homozygous NOD animals the immune response actively destroyed the β cells and thereby effected a diabetic condition (Prochazka et al. 1987). In this case, however, the autoantigen and its pattern of expression is unknown.

There are also indications that other variables may contribute to the induction of autoimmunity in the RIP-Tag transgenic mice. The case for factors in addition to MHC comes from the observation that every islet expresses the autoantigen and yet only a subset becomes infiltrated, and even then activation is neither immediate nor obligatory. Thus, one could infer that local differences among the 400 pancreatic islets may dictate their visibility to the immune system in both low and high responders. Among the possibilies are anatomical location within the pancreas and differences in the character of the islets, which do, for example, evidence both β-cell senescence and hyperplasia in a nonuniform manner in these mice (Teitelman et al. 1988). A second factor is implicated by the remarkably persistent failure to develop humoral autoimmunity in a majority of mice from the low responder (H-2^b) MHC background, which may belie significant differences in their immunological repertoire. For example, it is possible that a super antigen is inducing the clonal deletion of major classes of T-cell receptors during T-cell development. The absence of T cells bearing these receptors might be retarding effective activation of the autoimmune response when H-2^b molecules present T antigen. In this regard, it is of note that a recent study has revealed that H-2^b mice have very low levels of $CD4^+V_\beta 17a^+$ cells when compared with other haplotypes (Blackman et al. 1989). This observation is consistent with the notion that differences in deletion or positive selection could be manifesting the infrequent autoimmunity toward T antigen in the H-2^b background.

In summary, the interactions of T antigen with the immune system of transgenic mice present a model that is contributing to our understanding of the mechanisms by which immunological recognition of antigens expressed in rare cell types is achieved. The benefits of this system come from the existence of characteristic patterns of either developmental or delayed onset of expression among stable lineages of transgenic mice, the knowledge and accessibility of the self-antigen, and the implications that distinctive cellular interactions operate in the alternative induction of self-tolerance or autoimmunity.

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