Islet-specific T-cell clones from nonobese diabetic mice express heterogeneous T-cell receptors

(autoimmune disease/sequence/polymerase chain reaction)

SERGE CANDÉDIAS*, JONATHAN KATZ*, CHRISTOPHE BENOIST*, DIANE MATHIS*, and KATHRYN HASKINS†

*Laboratoire de Génétique Moléculaire du Centre National de la Recherche Scientifique et Unité 184 de Biologie Moléculaire de l'Institut National de la Santé et de la Recherche Médicale, Faculté de Médecine, Institut de Chimie Biologique, 11 rue Humann, 67085 Strasbourg, France; and †Barbara Davies Center for Diabetes, University of Colorado Health Sciences Center, 4200 East Ninth Avenue, Denver, CO 80262

Communicated by Philippa Marrack, April 16, 1991

ABSTRACT Nonobese diabetic (NOD) mice spontaneously develop a T-cell-mediated autoimmune disease that is similar in many respects to insulin-dependent diabetes mellitus in humans. T-cell clones that specifically recognize pancreatic islet cell antigens can be derived from NOD mice, and most of these have been diabetogenic upon transfer to healthy recipients. We report herein the sequences of the T-cell receptor α and β chains from four NOD-derived, islet-specific clones. The sequences are quite heterogeneous—in the junctional regions, specifically—so there seems to be little hope for treating this disease with specific anti-T-cell receptor reagents. This result contrasts with the strikingly restricted junctional region sequences reported for the receptors on clones derived from mice with experimental allergic encephalomyelitis, another T-cell-mediated autoimmune disease. We discuss possible explanations for this difference.

The precise molecular and cellular events leading to the development of T-cell-mediated autoimmune diseases have remained mysterious. Yet, our understanding of these maladies has advanced significantly over the past several years, and this increased comprehension has already led to proposals for new therapeutic strategies.

Experimental allergic encephalomyelitis (EAE) provides a good example. Injection of myelin basic protein (MBP) into certain mouse strains provokes a disease with many of the characteristics of multiple sclerosis in humans (for reviews, see refs. 1 and 2). MBP-reactive T cells can be cloned from diseased animals, and these are often capable of inducing EAE in naive recipients. When the T-cell receptors (TCRs) from Aμ-restricted, MBP-reactive clones were sequenced, they were found to be surprisingly homogeneous, using essentially only two Vβ and two Vα segments and only a limited set of J segments (V, variable; J, joining) (3, 4). Consequently, treatment with the appropriate anti-Vβ monoclonal antibody (mAb) could prevent (3, 4) or even reverse (3) disease, and immunization with the appropriate V or J region-derived peptides could vaccinate against EAE (5, 6). These findings have evoked much enthusiasm for anti-TCR-based therapeutic strategies, but one must ask how general they will prove to be.

We have addressed this question by sequencing TCRs from islet-specific T-cell clones derived from nonobese diabetic (NOD) mice. This strain spontaneously develops a disease with many similarities to human insulin-dependent diabetes mellitus (for review, see ref. 7). The implication of T cells in this disease is now indisputable, and it is possible to isolate clones that specifically recognize islet cell antigens and that promote diabetes on transfer to healthy recipients. We demonstrate that, in contrast to the situation with EAE, the

receptors on such clones are quite heterogeneous, suggesting that specific anti-TCR reagents may not be of much use in disease treatment.

MATERIALS AND METHODS

The islet-specific T-cell clones have already been described (8–10).

Details of the sequencing method will be reported elsewhere (S.C., unpublished data). Briefly, cytoplasmic RNA was prepared from 0.2–1.3 × 10⁷ cultured cells by the standard Nonidet P-40 lysis technique. This RNA was converted to cDNA by oligo(dT)-primed reverse transcription, and TCR α- or β-chain sequences amplified by two or three rounds of the PCR according to the strategy illustrated in Fig. 1A using the oligonucleotides listed in Fig. 1B. For the β chain, the first amplification was performed with an equimolar mixture of three degenerate V region oligonucleotides at the 5' end—NK121, NK122, and NK123—and a constant region oligonucleotide—MQ284—at the 3' end; the second amplification was with the same set of degenerate oligonucleotides at the 5' end and MS175 at the 3' end. For the α chain, the first amplification was performed with an equimolar mixture of three degenerate V region oligonucleotides at the 5' end—NW36, NW37, NW38—and a constant region oligonucleotide—NJ108—at the 3' end; second and third amplifications were with the same set of degenerate oligonucleotides at the 5' end and NJ109 or NJ110, respectively, at the 3' end. The PCR products were digested with Spal and EcoRI, enzymes for which recognition sequences had been incorporated via the primers used in the last round of PCR amplification—underlined sequences in Fig. 1B. The digested material was migrated on an agarose gel, the bands corresponding to specific products were cut out and eluted, and the purified fragments were cloned into an M13mp19 vector. Recombinant M13 plaques were detected by filter hybridization using OE7 as a probe for the β chain and OE6 for the α chain. Positive clones were sequenced by the dideoxynucleotide chain-termination method.

Elaborate precautions were taken to prevent sample contamination, a major problem of PCR-based techniques. All solutions were aliquoted, and aliquots were used only once. Aside from customary negative controls, a mock sample was processed along with each set of experimental samples—from the initial PCR through the screening of M13 plaques. This control ruled out contamination at any stage along the way.

RESULTS

Islet-Specific Clones. The Aμ-restricted, islet-specific T-cell clones have been described (8–10). They were derived

Abbreviations: NOD, nonobese diabetic; EAE, experimental allergic encephalomyelitis; MBP, myelin basic protein; TCR, T-cell receptor; mAb, monoclonal antibody; V, variable; J, joining.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. §1734 solely to indicate this fact.
**Fig. 1.** Sequencing strategy. (A) Placement of oligonucleotides. Products of reverse transcription of T-cell RNA were amplified by the PCR. NK121, -122, and -123 and NW36, -37, and -38 are degenerate oligonucleotides derived from the V regions of all β and α chains, respectively. They served as the 5' primer for all amplification. For the β chain, the 3' primers for the first amplification was MQ284 and for the second amplification it was MS175. For the α chain, the 3' primers were NJ108, -109, and -110, successively. The bar across some of the oligonucleotide arrows indicates an artificial restriction enzyme site introduced to facilitate cloning. OE7 and OE6 are the oligonucleotides used to screen for β- and α-chain clones, respectively. (B) Oligonucleotide sequences. The sequences of the oligonucleotides in A are presented. The NK and NW oligonucleotides are mixtures created during synthesis. The underlined bases are the restriction enzyme sites.

We also analyzed several of the other clones described previously (8-10) as well as some that have not been described. Unfortunately, firm assignments of TCR usage could not be made for any of these: some clones yielded exactly the same α- and β-chain sequences as BDC 6.9, which is not surprising given that they were isolated from the same line; others, presumably not fully clonal, gave rise to several different sequences, precluding unambiguous assignments. Among the latter, we found productively rearranged Vβ1, -6, -8.2, -8.3, -12, and -15 V regions. While inconclusive for the assignment of TCR usage, these data do serve to further emphasize the TCR heterogeneity we observed.

**DISCUSSION**

The TCR sequences described in this study are derived from T cells implicated in insulin-dependent diabetes mellitus. Their most noteworthy characteristic is their heterogeneity: no common features appear in the J regions of either the α or β chain (except perhaps for a paucity of charged amino acids). This finding is in concert with the preliminary immunohistological studies of H. Kikutani and T. Kishimoto (personal communication) showing that the islets of young NOD mice contain T cells that use diverse TCR Vβs. However, it is in contrast to a previous claim that the TCRs of a set of islet-specific T-cell clones predominantly bore Vβ5 V regions (14), as determined by staining with an anti-Vβ5 mAb, leading to the conclusion that diabetogenic T cells preferentially use Vβ5 TCRs. However, this conclusion has already been questioned because of results from transgenic mouse experiments (15). And Kikutani and Kishimoto (personal communication) have found that treatment of NOD mice with anti-Vβ5 mAb does not eliminate disease. While the data from spleen and lymph node preparations from newly diabetic, 3- to 5-month-old NOD mice and have been carried continuously on syngeneic islet cells and antigen presenting cells. Most of them—BDC 2.5, 5.2, and 6.9—have been demonstrated to be diabetogenic upon transfer into nonirradiated very young NOD mice (ref. 10; K.H., unpublished data); BDC 4.12 has not yet been tested. All four are CD4+.

The clones have all been analyzed with a protocol of specific anti-Vβ mAbs (K.H., unpublished data). BDC 2.5 and 6.9 were labeled with an anti-Vβ mAb, and BDC 5.2 was labeled with an anti-Vβ6 reagent. None of the mAbs in the panel reacted with BDC 4.12.

**TCR Sequences.** To obtain a complete description of the TCR α and β chains expressed by each clone, we sequenced mRNA transcripts by PCR technology. For each cell line, at least six α and nine β transcripts were analyzed, usually derived from several independent RNA preparations and PCR amplifications.

The sequences of the in-frame β-chain J regions are presented in Fig. 2A. Two of the T-cell clones express a TCR bearing the Vβ4 V region; otherwise there appear to be no shared sequence features. (BDC 4.12 also had an out-of-frame Vβ12 transcript.)

The sequences of the in-frame α-chain J regions are shown in Fig. 2B. Two of the clones express TCRs with V regions that are Vα13 family members and two other TCRs use the same Jα region. Otherwise, the clones display very heterogeneous TCRs, bearing quite different V and J regions. The BDC 2.5 and BDC 4.12 Vα sequences have not been described previously (they belong to the Vα1 and Vα13 families, respectively), so we present them more fully in Fig. 2C. (BDC 2.5 and 5.2 also had out-of-frame Vα1 and Vα8 transcripts, respectively.)
FIG. 2. Sequences of TCRs from islet-specific T-cell clones. The protein sequences of the β (A) and α (B) chains were derived from the nucleotide sequences. Only the CDR3 regions are presented. The underlined amino acids are from V- and J-region nucleotides. The following sources were used: Va4,19, ref. 11; Vβ13,1, ref. 12; all others, ref. 13. In C, we present more fully the sequences of the α chains for BDC 2.5 and BDC 4.12 because we have not found them in the literature.

The pronounced heterogeneity of islet-specific T lymphocytes in NOD mice is strikingly different from the near-homogeneity of MBP-specific T cells in mice with SLE, at least those of the H-2d haplotype (3, 4). In the latter disease, almost all T-cell clones reactive with MBP expressed one of two Vβ6 (Vβ8,2 or Vβ13) and one of two Vα5 (Vα9 or Vα4,2); in addition, these clones used a very restricted set of J segments, especially in the α chains. This near-homogeneity allowed the prevention (3, 4) or reversal (3) of SLE by treatment with an anti-Vβ6 mAb as well as vaccination against the disease by injection of V- and J-region-derived peptides (5, 6). These results have provided considerable enthusiasm for anti-TCR-based strategies as general palliatives for T-cell-mediated autoimmune diseases. However, it should be kept in mind that SLE is not a perfect model for such diseases in general or for multiple sclerosis in particular: it is induced experimentally by injecting large doses of a single peptide or protein antigen, and thus it might be expected to involve an unusually limited repertoire of autoreactive T cells. Indeed, a preliminary report described a very restricted Vα usage in brain lesions of multiple sclerosis patients (16), a more complete study has revealed much more diverse TCR profiles (17).

NOD, diabetes on the other hand, is a spontaneously arising malady and therefore is more representative of naturally occurring autoimmune diseases. Our results suggest that specific anti-Vβ reagents may not prove of much therapeutic value in these diseases—at least in insulin-dependent diabetes mellitus. Admittedly, the T-cell clones we examined were isolated from newly diabetic animals; hence, they were well into disease progression, when one would not be surprised to see a broad attack on islets by secondarily recruited T cells. In fact, the four clones studied here do not all appear to recognize the same islet-cell antigen (9). It is possible that disease-initiating T lymphocytes isolated at the earliest stages of islet infiltration might have a more limited repertoire and thus might be more prone to anti-Vβ intervention. Unfortunately, however, the diagnosis of human diabetes is also very late in the disease, when essentially all of the insulin-producing islet cells have already been destroyed, so at present, therapy aimed at disease-initiating lymphocytes appears not to be feasible.

In the end, the antigens that are the target for autoimmune attack will probably be found to behave similarly to classical foreign antigens. Reports have appeared describing foreign antigens that elicit T cells expressing restricted Vαs (18, 19) or restricted combinations of Vαs and Vβs (20–23); there have also been many examples of antigens eliciting heterogeneous populations of T cells (24–26). The effectiveness of anti-Vβ reagents for treating T-cell-mediated autoimmune diseases will no doubt depend on which category the target antigen...
falls into, and this could vary from disease to disease, as well as fluctuate with disease progression.

We would like to thank S. Vicaire, P. Bohn, and P. Gerber for excellent technical assistance, and A. Staub and F. Ruffenach for providing the oligonucleotides. This work was supported by institutional funds from the Institut National de la Santé et de la Recherche Médicale and Centre National de la Recherche Scientifique and by a grant to D.M. and C.B. from the National Institutes of Health (U.S.) and the Association pour la Recherche sur le Cancer. S.C. received fellowships from the Ministère de la Recherche et de la Technologie and the Association pour la Recherche sur le Cancer, and J.K. received a fellowship from the Human Frontiers Science Program.