adhesion molecules, namely ICAM-1 and ICAM-2. The mechanisms underlying the next step, transendothelial migration, are less clear but are assumed at the very least to involve a response to chemotactic stimuli. Our studies, along with other in vitro work (9), demonstrate that inhibition of PECAM-1 function can block the emigration of neutrophils and thus define the requirement for an additional cell adhesion molecule, PECAM-1, in the recruitment of neutrophils into inflammatory sites. It appears that this effect requires at least endothelial cell PECAM-1 because the anti–PECAM-1 used to block leukocyte transmigration in the human-SCID chimera model does not react against PECAM-1 on murine leukocytes (16). Although the mechanism by which PECAM-1 facilitates white blood cells through the endothelium is unresolved, blocking PECAM-1–mediated transmembrane migration of white blood cells may offer another target for therapeutic intervention in the treatment of inflammatory disorders.

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TECHNICAL COMMENTS

T Cell Receptor Specificity and Diabetes in Nonobese Diabetic Mice

In their search for the role of T cells in insulin-dependent diabetes mellitus (IDDM), Myra A. Lipes *et al.* (*1*) state that an intact T cell receptor (TCR) repertoire is not required for the generation of pancreatic β cell destruction in nonobese diabetic (NOD) mice. If IDDM is mediated by T cells, a single autoreactive TCR could lead to this disease. Therefore the key question is either, "What TCR genes are preferentially used?" or "Does T cell specificity play a role at all?" The report by Lipes *et al.* apparently does not address the first question, but does suggest that the TCR specificity of individual lymphocytes may not be essential for the amplification of the cell's lesions or for the development of insulitis. This finding seems to relate to the second question, yet their results do not convincingly support it.

Lipes *et al.* found that transgenic NOD mice bearing nondisease-related T cell receptor α and β subunit transgenes developed diabetes similar to that developed by control nontransgenic mice in terms of pathology and kinetics (*1*). T cell receptor α subunit transgene was transcribed, and β subunit transgene was expressed on the cell surface, which resulted in allelic exclusion on the endogenous TCRβ subunit gene locus (*1*). However, the expression of the TCRα subunit transgene (as determined by the amount of protein) is not shown; the presence of transcripts does not guarantee its expression on the cell surface. Therefore, it is possible that allelic exclusion on the TCRα endogenous gene locus did not occur (*1*). As a result, autoreactive TCRs may have been produced from the gene rearrangement. Another study suggests (*2*) that even though α transgene is expressed on the cell surface of T lymphocytes, rearrangement on the endogenous TCRα locus could still occur if T lymphocytes (bearing the TCR encoded by the transgenes) cannot be positively selected during their development. Lipes *et al.* do not address the question of whether the TCR-encoded transgene was compatible with the major histocompatibility complex (MHC) molecules of NOD mice, and thus, whether T cells bearing this TCR could be positively selected. Moreover, information about the specificity of T cells carrying transgenic TCR molecules was not provided. Despite the fact that both α and β subunit transgenes were derived from nondisease-related TCRs, the specificity after pairing them together is not known. Thus, one cannot rule out the possibility that T cells bearing transgene-encoded TCR could attack β cells of pancreas islets; this could be another source for the TCR specificity required for disease generation.

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Research on juvenile diabetes and the NOD mouse model on which it is based are challenged in the report by Lipes *et al.* (*1*). They found that NOD mice expressing one random pair of transgenes for the α and β chains of the TCR (consisting of the α chain from an anti–L^d $CD8^+$ clone and the β chain from an anti–chicken ovalbumin $CD4^+$ clone) have lymphocytic infiltration of their pancreas and the same incidence of diabetes as the nontransgenic NOD mice. Lipes *et al.* suggest that the T cells of these transgenic mice bear exclusively the transgenic TCRαβ and conclude that "the TCR specificity of individual lymphocytes may not be essential." In other words, T cells, irrespective of the specificity of their TCR, are driven to participate in the selective destruction of the β cells that produce insulin inside the islets of Langerhans. The supposition that T cells could be involved in immune responses where their TCR is not engaged has major theoretical implications that are not discussed in the paper. How, for example, could tolerance to self antigens be imparted if the antigen specificity of TCRs could be bypassed for T cell activation?

There is a simple way to account for the results described by Lipes *et al.* without conflicting with basic immunological concepts or with the widely accepted view that diabetes results from a T cell–mediated autoimmune process specifically targeting β cell antigens. When a pair of transgenic rearranged α and β

TCR chain genes is introduced into a particular mouse strain, the TCR repertoire selected in the thymus may be of two types: it may consist of (i) a quasi-exclusive representation of the transgenic TCRαβ or (ii) a mixture of the transgenic TCRαβ ($\alpha_T\beta_T$) and TCRs comprised of the transgenic β chain and one of several endogenous α chains ($\alpha_E\beta_T$). The latter situation occurs when the transgenic $\alpha_T\beta_T$TCR cannot be selected by the set of MHC molecules of the particular mouse strain in which it has been introduced (*2*), and is made possible by the fact that endogenous α chain genes, in contrast with endogenous β chain genes, are not allelically excluded by the rearranged transgenes. Such a repertoire, although it is obviously not as broad as that of nontransgenic mice, can still, in theory, consist of a wide set of TCRs, on the basis of junctional and V (variable) region diversity in the α chain. This expression of endogenous TCRα chains was examined by anchored polymerase chain reaction (PCR) in messenger RNA (mRNA) by Lipes *et al.* (*1*). They found that only 17% of the C_α-positive complementary DNA (cDNA) plasmid clones were derived from endogenous α transcripts; however, it was not determined whether the transgenic α chain protein was represented at the surface of the cell. It is conceivable that the transgenic α and β proteins do not pair well and so do not get to the surface. TCRs containing endogenous α chain proteins might be the dominant receptors on the surface even though the transgenic α mRNA is a major species in the cytoplasm. Thus, there is no way to tell (at the moment) what type of TCR repertoire is available in these transgenic mice. Little is known about the selection pattern of the particular transgenic $\alpha_T\beta_T$ TCR used by Lipes *et al.*, and the second phenotype ($\alpha_E\beta_T$) may prevail in the transgenic NOD mice, as a result of the relative stringency of the positive selection phenomenon.

If this is indeed the phenotype observed in the αβTCR transgenic NOD, the results by Lipes *et al.* are compatible with the view that the specificity of individual TCRs plays a major role in the β cell destruction process observed in NOD mice. A simple prediction of this view is that introducing the *scid* or the *RAG-1* or *RAG-2* mutations into these TCR transgenic mice, in order to reduce or abolish the endogenous αTCR chain gene rearrangements, would completely suppress both insulitis and diabetes.

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Lipes *et al.* (*1*) conclude that the role of T lymphocytes in the pathogenesis of diabetes may not be directly linked to their antigenic specificities. Rather, T cells might only be required for a secondary, nonspecific amplification of a primary lesion. This suggestion departs from the commonly accepted notion that direct recognition of pancreatic β cell antigens by T cells is central to the pathogenesis of diabetes (*2*). The overturning of established dogma is part of scientific progress, but requires indisputable data, which have not been provided in this case.

Two lines of transgenic mice were examined in this paper. The first expressed a rearranged TCR β gene derived from a chicken conalbumin-specific T cell line. These mice had a somewhat skewed repertoire, as all T cells used the same TCR β chain, but still had a significant degree of diversity as a result of extensive α-chain variability. Thus, diabetes in NOD mice is not due to a highly restricted combination of TCR α and β chains.

The second line of mice carried rearranged transgenes for both the α and β TCR chains. It is the interpretation of these data with which we take issue. Lipes *et al.* suggest that the αβ transgenic was an essentially monoclonal mouse and, because it was as susceptible to diabetes as unmanipulated NOD mice, proposed that the specificity of the T cells is of secondary importance for pathogenesis. However, the expression of endogenous TCR α chains needs closer examination. The α transgene was expressed as RNA in many T cells. But it has been found in many systems that the presence of a transgenic α chain does not prevent rearrangement and use of the endogenous α chain genes (*3*). Many of the peripheral T cells in an H-Y TCR transgenic mouse do not display the transgene-encoded α chain at their surface, but they all express transgene-encoded α chain transcripts (along with endogenous α chain transcripts) (*3*). Lipes *et al.* (*1*) do not document the use of endogenous α chains in their mice, but other studies indicate that TCR-αβ transgenic mice probably display a highly diverse repertoire of α chains.

This prediction is made even more likely by the choice of genes in their study (*1*): The α chain transgene originated from an MHC class I–restricted T cell that reacts specifically with the L^d molecule, and the β chain transgene was obtained from a T cell clone restricted by the MHC class II molecule A^b that reacts specifically with chicken conalbumin. It is unlikely that this improbable combination would just happen to have affinity for either class I or class II (MHC) molecules of the NOD haplotype. Thus, the positive selection events needed to generate $CD4^+$ and $CD8^+$ T cells in these αβ transgenic mice likely require the participation of endogenous α chains. On the basis of the data presented (*1*), these TCRαβ mice do not appear to be significantly different from the TCRβ transgenic mice, and any additional conclusions seem unwarranted.

The means to resolve this issue are available. The most convincing experiment would be to cross the transgene onto a background deficient in RAG, which would eliminate the contribution of endogenous TCR genes. However, this is a long-term experiment. In the meantime, one could provide two other important pieces of information.

First, what do the CD4 and CD8 profiles look like in the αβ transgenics? If the two transgene-encoded chains combine to form a specificity that is selected by NOD MHC molecules, the distribution of mature T cells should be skewed into either the CD4 or CD8 compartment. This skewed distribution should be absent or less evident in the TCRβ transgenic mice and is unlikely to be observed in the absence of the NOD MHC. Lack of a skewed distribution to either the CD4 or CD8 compartment would be more consistent with a heterogeneous α-chain contribution.

Second, does allelic exclusion of endogenous α chain genes actually occur in these αβ transgenics? This would be easy to test with the use of the several antibodies to TCR Vα reagents that are widely available. To our knowledge, complete allelic exclusion of endogenous α chain genes has not been observed with the use of these reagents in other TCRαβ transgenics.

In summary, Lipes *et al.* found a normal occurrence of diabetes in NOD mice with skewed (but still highly diverse) T cell repertoires. These data are interesting in and of themselves, but do not warrant the conclusion (*1*) that the specificity of the lymphocytes is not important for pathogenicity.

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Response: Although it is widely established that type I diabetes is T cell–dependent, the nature of the target autoantigen (or autoantigens) and the precise mechanism (or mechanisms) by which the β cell is specifically destroyed are unknown. According to one scheme of diabetes pathogenesis, $CD4^+$ T cells function as helper cells for the activation of $CD8^+$ T cells that damage β cells by a direct cytotoxic attack (*1*). This classical model implies that specific cytotoxic $CD8^+$ T cells directly attack islet cells, and it provides an explanation for the selectivity of the process of β cell destruction and for the dual requirement for $CD4^+$ and $CD8^+$ cells in the initiation of disease. However, more recent studies have demonstrated that islet damage may not be the result of a direct interaction between $CD8^+$ T cells and the target β cell (*2*). On the basis of these findings, it has been proposed that β cell killing occurs through an "indirect pathway" from a nonspecific inflammatory response that initially involves $CD4^+$ cells. Finally, it is possible that macrophage infiltration itself may be directly responsible for the dysfunction and death of the β cells through the release of cytokines and free oxygen radicals that may be selectively cytotoxic to β cells (*3*). In this latter scenario, functional T cells would still be recruited to the lesion, but the specificity of individual cells would not be essential.

The literature on NOD mice has emphasized the antigen-driven etiology of diabetes and intensive efforts have been made trying to determine whether a restricted TCR repertoire is required for the initiation of the process. TCR repertoire restriction would suggest that the presence of a single (or few) peptide epitopes of a single antigen would result in the expansion of T cells bearing specific TCR sequences that are critical to the pathogenesis of disease. Our aim in these experiments (*4*) was to determine whether autoimmunity would be influenced in transgenic NOD mice that were capable of expressing predominantly disease-unrelated TCR β and αβ chain genes. We reasoned (*4*) that if, in diabetes, pathogenic T cells recognized a very limited set of peptide epitopes, autoantigen recognition would depend on a few specific αβ TCRs and that depleting these receptors would have protected against disease.

Our transgenic studies (*4*) indicated that, at a minimum, the development of diabetogenic T cells in NOD mice was the result of a redundant T cell repertoire that was not defined by particular TCR αβ combinations. Our studies suggest that the exact specificity of the TCR β chain may be unimportant and that T cells bearing a multitude of β chains, including an antigenically irrelevant transgenic β chain, might cause disease.

Although Benoist and Mathis suggest that the ratio of CD4 to CD8 should have been preserved in our single β chain mice and skewed in our αβ transgenic mice, we have found that the opposite occurred. The CD4/CD8 ratio was skewed toward $CD8^+$ in our single TCR β chain mice (1.0 ± 0.11 SEM, $n = 3$), whereas in the double αβ chain NOD mice the ratio was normal (2.0 ± 0.20 SEM, $n = 3$) and similar to that in nontransgenic controls (2.3 ± 0.17 SEM, $n = 4$). We know of no studies of TCR transgenic mice in which the α and β chains have been derived from T cell clones of different antigen and MHC specificities, and it is difficult to predict in which direction (CD4 or CD8), if any, T cell development would be biased. Our TCR αβ transgenic NOD mice differ from our single β chain transgenic NOD mice, which suggests (but does not prove) a functional effect of the α chain transgene.

Although we could demonstrate that the β chain transgene was expressed on the vast majority of T cells, we did not have an antibody that specifically recognized our transgenic α chain, nor was it technically possible to determine the α chain transgene surface expression or the diversity of the α chain repertoire by biochemical techniques, as we discussed (*4*). The few V_α monoclonal antibodies that were available were not useful because of the low (<1%) baseline expression of these V_α families in control NOD mice. We therefore quantitated the endogenous TCR repertoire in lymph node mRNA by anchored PCR. These studies revealed that 83% of the C_α positive plasmid colonies expressed the Vα3.1 transgene (Vα3.1 was not detectable in nontransgenic control NOD mice). To examine these frequencies at a cellular level, we generated a large panel of hybridomas by fusing splenic T cells from our transgenic and nontransgenic mice with the TCR $(\alpha\beta)^-$ thymoma cell line (*4*). These studies showed transgenic α chain frequencies (86%) similar to those in anchored PCR and demonstrated the consistent coexpression of TCR α and β transgenes within individual cells (*4*). Although our transcriptional data are consistent with abundant surface expression, the presence of transcripts may not guarantee cell surface expression [as our colleagues point out in regard to a recent paper (*5*)]. Definitive resolution of the issue of surface expression of the α chain transgene, however, awaits long-term studies to breed the RAG mutation onto the NOD background.

Our data, as we have stated (*4*), suggests that markedly skewing the TCR repertoire may not diminish the progression to autoimmunity in NOD mice. We did not state that "the T cells of these transgenic mice bear exclusively the transgenic TCRαβ . . ." or that we have generated a "monoclonal" mouse. Because ablating totally the expression of endogenous TCR genes by the transgenic approach is not possible, we are deriving RAG-deficient αβ TCR transgenic NOD mice. The analysis of these mice, devoid of endogenous TCR rearrangements, may enable us to further define the TCR requirements for the initiation of islet autoimmunity.

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