The Absence of a Glycemic Threshold for the Development of Long-Term Complications: The Perspective of the Diabetes Control and Complications Trial

The Diabetes Control and Complications Trial (DCCT) demonstrated a reduction in the development and progression of the long-term complications of IDDM with intensive therapy aimed at achieving glycemic control as close to the nondiabetic range as possible. The DCCT subsequently showed that the total lifetime exposure to hyperglycemia was the principal determinant of the risk of retinopathy and that there was a continuous nonlinear relationship between this risk and the mean level of HbA$_1c$ (DCCT Research Group, Diabetes 44:968-983, 1995). In contrast, other authors, based on a retrospective study (Krolewski et al., N Engl J Med 332:1251-1255, 1995), have suggested that a glycemic threshold for microalbuminuria and retinopathy exists at an HbA$_1c$ level of ~8%, below which there is no further appreciable reduction in risk. In this perspective, we examine whether the DCCT data demonstrate such a glycemic threshold for the development of retinopathy, nephropathy, or neuropathy. In the DCCT, 1,441 patients with IDDM were randomly assigned to intensive (a = 711) or conventional (a = 730) therapy and followed for a mean of 6.5 years. Retinopathy was assessed every 6 months by stereoscopic fundus photography; albumin excretion was measured annually in a 4-h collection; and neuropathy was assessed with a standardized protocol performed at baseline and at 5 years. Glycosylated hemoglobin was measured quarterly. Episodes of severe hypoglycemia were ascertained using standardized procedures. The risks (hazard rates) of retinopathy progression and of developing microalbuminuria and neuropathy were found to be continuous but nonlinear over the entire range of glycosylated hemoglobin values in the intensive, conventional, and combined treatment groups. These nonlinear relationships describe a constant relative risk gradient in which proportional reductions in HbA$_1c$ are accompanied by proportional reductions in the risk of complications. Although the magnitude of the absolute risk reduction declines with continuing proportional reductions in HbA$_1c$, there are still meaningful further reductions in risk as the HbA$_1c$ is reduced toward the normal range. When the instantaneous risks for different complications associated with different HbA$_1c$ values are compounded over time, there are substantial differences in the cumulative incidence of patients experiencing a complication for patients with HbA$_1c$ values of 6 vs. 7 vs. 8% or higher. In fact, no HbA$_1c$ threshold could be identified, short of normal glycemia, below which there was no risk of the development or progression of these complications. Furthermore, as the HbA$_1c$ was reduced proportionately, the proportional rate of decline in the relative risk for each of these complications was similar for HbA$_1c$ levels £8.0% and for levels >8%. In contrast, although the absolute risk of severe hypoglycemia in the intensive treatment group increased as the HbA$_1c$ decreased, the relative risk gradients were significantly less for HbA$_1c$ levels £8.0% than for levels >8%.

These extensive prospective DCCT data do not support the conjecture that a glycemic threshold for the development of complications exists at an HbA$_1c$ of 8% or that an HbA$_1c$ goal of 8% is maximally beneficial. In the DCCT, as HbA$_1c$ was reduced below 8% there were continuing relative reductions in the risk of complications, whereas there was a slower rate of increase in the risk of hypoglycemia. Therefore, the DCCT continues to recommend implementation of intensive therapy with the goal of achieving normal glycemia as early as possible in as many IDDM patients as is safely possible. Diabetes 45:1289-1298, 1996

The Diabetes Control and Complications Trial (DCCT) was a multicenter randomized controlled clinical trial that demonstrated that, compared with conventional diabetes therapy, intensive therapy reduced by 35 to 75% the development and progression of the long-term complications of IDDM (1,2). The goal of intensive therapy was to achieve glycemic levels in the nondiabetic range (HbA$_1c$ <6.05%);
the mean HbA1c actually achieved with intensive therapy was 7.2% during the study, ~2 units lower than that achieved with conventional therapy. These reductions in HbA1c were accompanied by a threefold increase in the risk of severe hypoglycemia with intensive versus conventional treatment (2,3).

The primary analysis of the DCCT results, as mandated by the study design, compared the long-term outcomes of the two randomly assigned treatment groups. The DCCT also reported a detailed epidemiological analysis of the association between glycemic levels and risk of retinopathy progression observed in the DCCT (4). This analysis demonstrated a continuously increasing risk of retinopathy progression with increasing mean glycosylated hemoglobin level. Earlier analyses also showed that the risk of severe hypoglycemia within the intensive treatment group increased apparently exponentially as the HbA1c was reduced (2).

Based on the initially published DCCT results (2), some have recommended that an HbA1c of ~7%-8% should be a goal for treatment that would achieve a maximal benefit-risk ratio, weighing the reductions in the risk of complications with the increased risk of hypoglycemia (5). Further, a recently published study (6), based on a retrospective analysis of urine albumin measurements and glycosylated hemoglobin levels measured 1 and 3 years before the renal assessment, reported that the risk of microalbuminuria “was almost flat” or nearly constant for levels of HbA1c <10.1% (estimated to be comparable to an HbA1c <8.1%). This was interpreted to suggest that a biological threshold for risk existed at an HbA1c of ~8%. These authors concluded that little or no clinical benefit would result from a further decrease below this level (6).

The article, an accompanying editorial (7), and a letter to the editor that suggested a similar glycosylated hemoglobin threshold for progression of retinopathy (8), recommended that therapy of IDDM be directed at achieving HbA1c levels <8.1%, as opposed to the HbA1c goals of intensive therapy as implemented in the DCCT.

These recommendations prompted us to compare the associations of HbA1c values ≤8% vs. those >8% with the risk of development of nephropathy (microalbuminuria) and neuropathy, with progression of retinopathy, and with the incidence of severe hypoglycemia. These analyses were performed within the intensive and conventional treatment groups of the DCCT and for both groups combined and are based on extensive DCCT data collected prospectively during >9,000 patient-years of observation.

**RESEARCH DESIGN AND METHODS**

In the DCCT, 1,441 subjects were recruited at 20 clinical centers during 1983–1989 and were followed for an average of 6.5 years. Patients were recruited either within a primary prevention cohort (n = 726) consisting of patients with 1–5 years’ duration of IDDM, no retinopathy detected by seven-field stereoscopic fundus photography, and urinary albumin excretion <40 mg/24 h, or within a secondary intervention cohort. The secondary intervention cohort comprised 715 patients with 1–15 years’ duration of IDDM, mild or nonproliferative diabetic retinopathy, and urinary albumin excretion 50–450 mg/24 h. Of these, 139 patients entered the study with advanced microalbuminuria.

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inherently adjust for the background variation in risk over time were also used. PH models of retinopathy progression were stratified-adjusted for the baseline level of retinopathy; models of nephropathy onset and progression were adjusted for the log(AER) at baseline and stratified by primary versus secondary cohort. A generalization of the PH model for recurrent events, the multiplicative intensity model (MIM) (19), was used for the analysis of hypoglycemic events. These PH (MIM) models provided similar results to those of the simpler Poisson models, only the Poisson models are presented. The logistic regression model (18) was used to describe the association between the updated log(mean HbA1c) at 5 years and the risk (odds ratio) of confirmed clinical neuropathy at 5 years. Models in the combined groups were adjusted for intensive versus conventional treatment by further stratification in the PH models or by adding an additional term in the Poisson and logistic models.

All analyses used the natural log of the updated mean HbA1c, which provided a better fit than using the mean HbA1c itself (4). These regression models express the log(risk) as a linear function of the log(HbA1c), i.e., a straight line is used to describe the relationship between the log(risk) versus the log(HbA1c). To assess the validity of this log-log model, a nonparametric (model-free) spline function was fit to the raw data with these log transformations. The results were strongly linear, indicating that this model is appropriate.

From a linear log-log relationship, the risk gradient can be expressed as the percentage reduction in risk for a fixed 10% reduction in the HbA1c, computed as (0.9\(^{-\beta} - 1 \times 100, where β is the coefficient (slope) for log(HbA1c). Such models describe a constant relative risk relationship over the range of HbA1c. A continuous nonlinear absolute risk relationship for the risk versus HbA1c, termed the simple exponential risk gradient, is obtained when a straight line fit to the log(risk) versus the log(HbA1c) is presented in the original scales of measurement (untransformed).

A change point is defined as a value of HbA1c below and above which there is a statistically significant difference in the slope or risk gradient for the association with the risk of complications. The presence and significance of a change point was assessed through segmented log(risk) versus log(HbA1c) regression models that included a linear predictor of the form \( \beta_1 (x) + \beta_2 (x - c)I(x > c) \), where \( x \) is the log(mean HbA1c), \( I(.) \) is the (0,1) indicator function, and \( c \) is the assumed change point. With \( c = \log(8) \), this model provides an estimate of the risk gradient for HbA1c values \( \leq 8% \) (\( \beta_1 \)) and the risk gradient for values \( >8% \) (\( \beta_2 + \beta_1 \)). To test whether a change point exists at the specified value (c), the improvement in fit of the segmented regression model over the simple exponential model was assessed by a likelihood ratio test comparing this model with a model containing the \( \beta_1 \) effect alone, which is also a test that \( \beta_2 = 0 \). A test for an optimal change point selected by a search among a range of values from 0 to 9.9% in increments of 0.1% was performed using a 2 df likelihood ratio test of the segmented model at the optimal value compared to the simple exponential model (20).

A threshold is defined as a change point below which the risk, if any, is constant, or the risk gradient is zero. A test of a threshold at a specified change point (c) was assessed by a Wald test that \( \beta_2 = 0 \) (20).

Poisson regression analyses within the intensive plus conventional groups combined were adjusted for treatment group. To describe the risk gradients in the figures, the treatment group effect was absorbed into the intercept (\( \alpha \)) assuming half the patients were in each group.

These risk gradients describe the instantaneous hazard rate (\( \lambda \) per year) of events at any point in time as a function of the current updated log(mean HbA1c) (\( x \)) of the form \( \lambda(x) = \exp(\alpha + \beta x) \). For an individual patient with a constant log(mean HbA1c) of \( x \) during the study, the cumulative incidence of an event is estimated from the integrated cumulative hazard over the 9 years of follow-up in the DCCT as \( 1 - \exp(-\Lambda(9\times x)) \).

All results nominally significant at \( P < 0.05 \) are indicated.

RESULTS

Intensive versus conventional treatment effects.

The distribution of the mean HbA1c over the total period of follow-up of each subject, ranging from 4 to 9 years, in the conventional and the intensive treatment groups, is shown in Fig. 1. (A similar figure, but incorporating the mean HbA1c up to each semi-annual visit, is presented in Ref. 4.) In the conventional treatment group, the median value at the final visit was 9.02 and only 20.3% of patients (\( n = 148 \)) had a mean HbA1c of 8 or less. In the intensive treatment group, the median of the values at the final visit was 7.07, and 83.4% of patients (\( n = 593 \)) had a mean HbA1c of 8 or less. In the combined conventional plus intensive groups, approximately equal numbers of patients had a mean HbA1c ≤8 (\( n = 741 \)) and >8 (\( n = 700 \)). Thus, maximal power to distinguish a difference in risk over the range of HbA1c, values ≤8 versus that among values >8 is provided by an analysis among the combined treatment groups.

Previously, the DCCT showed that intensive treatment, compared to conventional treatment, reduced the risk of retinopathy by 63% and of microalbuminuria by 39% in the combined cohorts (2). The less striking effect of intensive treatment on the risk of microalbuminuria, compared with retinopathy, is attributed to the greater degree of random within-patient variation for this outcome measurement. Analyses using less variable outcomes (14) revealed that intensive treatment reduced the risk of developing sustained microalbuminuria by 60% and of developing advanced microalbuminuria by 51%. Therefore, the relationship between nephropathy and HbA1c was explored using these other more stable manifestations of nephropathy as well as microalbuminuria.

Exponential risk gradients. For each complication, Table 1 presents the percentage reduction in the instantaneous hazard rate (the risk gradient) associated with a 10% reduction in HbA1c. The risk gradient value is obtained from the slope of the linear relationship of the log of risk versus the log of the mean HbA1c, which is a simple exponential model. Values are presented for analyses among the intensive and conventional treatment group patients separately and also for an analysis of the combined intensive plus conventional treatment groups. The risk gradients for all complications were of similar magnitude in the intensive and conventional groups and were not significantly different between the treatment groups for any complication. Thus, it was reasonable to examine the HbA1c risk gradient without regard to treatment.

For each retinal, renal, and neurological complication assessed, there was a significant reduction in risk (the
TABLE 1
Relative risk reductions associated with a 10% lower mean HbA1c in the intensive and conventional treatment groups and in the combined groups

<table>
<thead>
<tr>
<th>Retinopathy (final ETDRS)</th>
<th>Intensive therapy</th>
<th>Conventional therapy</th>
<th>Combined groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sustained onset, primary cohort only</td>
<td>39 (25–49)*</td>
<td>34 (26–41)*</td>
<td>35 (29–41)*</td>
</tr>
<tr>
<td>Sustained progression (≥3 steps)</td>
<td>43 (33–51)*</td>
<td>37 (31–43)*</td>
<td>39 (34–44)*</td>
</tr>
<tr>
<td>SNPDR (≥level 53), secondary cohort only</td>
<td>26 (2–44)±</td>
<td>41 (30–50)*</td>
<td>37 (27–45)*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nephropathy</th>
<th>Intensive therapy</th>
<th>Conventional therapy</th>
<th>Combined groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microalbuminuria (≥40 mg/24 h)</td>
<td>23 (17–37)*</td>
<td>24 (15–32)*</td>
<td>25 (19–32)*</td>
</tr>
<tr>
<td>Advanced microalbuminuria (≥100 mg/24 h)</td>
<td>44 (29–55)*</td>
<td>35 (23–46)*</td>
<td>39 (29–47)*</td>
</tr>
<tr>
<td>Albuminuria (≥300 mg/24 h), secondary cohort</td>
<td>36 (19–49)*</td>
<td>36 (32–50)*</td>
<td>36 (26–45)*</td>
</tr>
<tr>
<td>Neuropathy</td>
<td>Intensive therapy</td>
<td>Conventional therapy</td>
<td>Combined groups</td>
</tr>
<tr>
<td>Confirmed clinical neuropathy</td>
<td>32 (9–50)±</td>
<td>29 (15–41)*</td>
<td>30 (18–40)*</td>
</tr>
</tbody>
</table>

Data for the combined groups are from a Poisson regression model adjusted for intensive vs. conventional treatment. *P < 0.001. †P < 0.01. ‡P < 0.05.

hazard rate) associated with a 10% reduction in HbA1c over the entire range of HbA1c. The risk gradient for microalbuminuria was less steep than that of the other renal manifestations owing to the greater degree of random within-patient variation. Results from PH models were similar to those presented in the table.

The linear regression of the log of the risk (hazard rate) of sustained progression of retinopathy versus the log of

![Image](https://example.com/image.png)

**FIG. 2.** The absolute risk of sustained retinopathy progression (hazard rate per 100 patient-years) in the combined treatment groups as a function of the updated mean HbA1c during follow-up in the DCCT estimated from a Poisson regression model with 95% confidence bands: α = −13.45, β (coefficient for log HbA1c) = 4.68, and offset = log(1/2 year). A: log(rate) vs. log(HbA1c). B: rate vs. HbA1c over the range observed in the trial. C: rate vs. values of HbA1c ≤8%. D: cumulative incidence (probability) over 9 years of treatment vs. HbA1c.
FIG. 3. The absolute risk of microalbuminuria (hazard rate per 100 patient-years) in the combined treatment groups as a function of the updated mean HbA1c during follow-up in the DCCT estimated from a Poisson regression model with 95% confidence bands: $\alpha = -9.18$; $\beta$ (coefficient of log HbA1c) = 2.79, and offset = log(1 year).

A: log(rate) vs. log(HbA1c). B: rate vs. HbA1c over the range observed in the trial. C: cumulative incidence (probability) over 9 years of treatment vs. HbA1c.

The mean HbA1c is shown in Fig. 2A for the combined treatment groups. As the log of the HbA1c decreased, the log of the risk also decreased in a strongly linear fashion, as indicated by the log of the crude rate within 1/12th percentiles of the HbA1c values. Note also that among the lower levels of HbA1c, it was difficult to estimate the shape of the relationship precisely because the rates were estimated with less precision, as indicated by the larger standard error bars, owing to the smaller numbers of events at lower HbA1c values. Nevertheless, this figure shows that there is a continuing decline in risk with lower HbA1c.

Figure 2B presents the same relationship expressed in the original units of measurement showing the absolute risk (rate per 100 patient-years) versus the updated mean HbA1c. From the estimated linear log-log relationship, for each 10% reduction in HbA1c, such as from 9 to 8.1 or from 7 to 6.3, there was a constant 30% reduction in risk. As the HbA1c values were reduced proportionately, the reductions in absolute risk became smaller because the risk relationship was based on a constant relative or percentage reduction in risks. Thus, we observed a continuous simple exponential nonlinear risk relationship. Figure 2C presents the same risk gradient, but only over the range of HbA1c values ≤8%. With a rescaling of the y-axis, this clearly shows the continuing reduction in risk as the HbA1c values were reduced below 8% and the absolute risk became smaller.

Figures 2B and 2C describe the instantaneous hazard rate of retinopathy progression as a function of the updated HbA1c. For any given value of HbA1c assumed held constant over time, the cumulative incidence of retinopathy progression can then be estimated by compounding these instantaneous risks over the 9 years of follow-up in the DCCT, as shown in Fig. 2D over the complete range of HbA1c values. These figures show that small differences in the instantaneous risks (Fig. 2B) correspond to larger differences in the total probability (cumulative incidence) over a period of many years.

Similarly, Fig. 3A presents the estimated linear relationship between the log of the risk (hazard rate) of developing microalbuminuria and the log of the current HbA1c within the combined intensive plus conventional treatment groups. Across the range of HbA1c values, the relationship is linear, but again it is difficult to estimate precisely the nature of the relationship among lower values of the HbA1c, because of imprecision in the estimates of the rates.

Figure 3B presents the exponential relationship between the risk of developing microalbuminuria and the current mean HbA1c, without the log transformations. For each 10% reduction in the HbA1c, there was a 25% reduction in risk. Figure 3C presents the estimated cumulative incidence as a function of a constant mean HbA1c value over the 9 years of follow-up in the DCCT.

Threshold and change point models for complications. Relative risk gradients associated with a 10% reduction in HbA1c were obtained from segmented Poisson regression models with a change point at an HbA1c of 8% (Table 2). These models provide separate estimates for the slope of the regression of the log(risk) on the log(HbA1c) for HbA1c values ≤8 and values >8. From these log-log regression model slope estimates, separate estimates of the relative risk gradient were calculated for a 10% reduction in the updated mean HbA1c over the range of HbA1c values ≤8 and over the range of HbA1c values >8.

In general, all of the complications examined, including retinopathy progression, microalbuminuria, sustained microalbuminuria, and confirmed clinical neu-
TABLE 2
Relative risk reductions associated with a 10% lower mean HbA\textsubscript{1c} among HbA\textsubscript{1c} values ≤8 vs. values >8% estimated from a segmented (change point) model

<table>
<thead>
<tr>
<th>Complication</th>
<th>HbA\textsubscript{1c} ≤8%</th>
<th>% risk reduction</th>
<th>95% CI</th>
<th>P</th>
<th>HbA\textsubscript{1c} &gt;8%</th>
<th>% risk reduction</th>
<th>95% CI</th>
<th>P</th>
<th>P for HbA\textsubscript{1c} ≤8 vs. &gt;8%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sustained retinopathy progression</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intensive</td>
<td>49</td>
<td>0.0003</td>
<td>(27-65)</td>
<td>0.0003</td>
<td>37</td>
<td>(17-53)</td>
<td>0.002</td>
<td>0.46</td>
<td></td>
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<tr>
<td>Conventional</td>
<td>69</td>
<td>0.008</td>
<td>(29-87)</td>
<td>0.0001</td>
<td>34</td>
<td>(26-41)</td>
<td>&lt;0.0001</td>
<td>0.055</td>
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<tr>
<td>Intensive and conventional</td>
<td>53</td>
<td>&lt;0.0001</td>
<td>(37-66)</td>
<td>&lt;0.0001</td>
<td>35</td>
<td>(28-42)</td>
<td>&lt;0.0001</td>
<td>0.052</td>
<td></td>
</tr>
<tr>
<td>Microalbuminuria</td>
<td></td>
<td></td>
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<tr>
<td>Intensive</td>
<td>23</td>
<td>0.036</td>
<td>(2-40)</td>
<td>0.005</td>
<td>33</td>
<td>(11-50)</td>
<td>0.006</td>
<td>0.55</td>
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<td>Conventional</td>
<td>21</td>
<td>0.37</td>
<td>(-31-52)</td>
<td></td>
<td>24</td>
<td>(13-34)</td>
<td>&lt;0.0001</td>
<td>0.87</td>
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<tr>
<td>Intensive and conventional</td>
<td>25</td>
<td>0.098</td>
<td>(8-39)</td>
<td></td>
<td>26</td>
<td>(16-34)</td>
<td>&lt;0.0001</td>
<td>0.97</td>
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<tr>
<td>Sustained microalbuminuria</td>
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<tr>
<td>Intensive</td>
<td>43</td>
<td>0.041</td>
<td>(2-67)</td>
<td>0.005</td>
<td>44</td>
<td>(17-62)</td>
<td>0.006</td>
<td>0.97</td>
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<tr>
<td>Conventional</td>
<td>58</td>
<td>0.18</td>
<td>(-50-88)</td>
<td></td>
<td>33</td>
<td>(17-45)</td>
<td>0.0002</td>
<td>0.47</td>
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<tr>
<td>Intensive and conventional</td>
<td>49</td>
<td>0.005</td>
<td>(19-68)</td>
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<td>35</td>
<td>(23-46)</td>
<td>&lt;0.0001</td>
<td>0.40</td>
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<tr>
<td>Confirmed clinical neuropathy</td>
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<tr>
<td>Intensive</td>
<td>30</td>
<td>0.19</td>
<td>(-19-58)</td>
<td>0.16</td>
<td>35</td>
<td>(-17-64)</td>
<td>0.87</td>
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<td>Conventional</td>
<td>32</td>
<td>0.41</td>
<td>(-70-73)</td>
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<td>28</td>
<td>(11-43)</td>
<td>0.003</td>
<td>0.93</td>
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<tr>
<td>Intensive and conventional</td>
<td>32</td>
<td>0.082</td>
<td>(-5-56)</td>
<td>&lt;0.0008</td>
<td>29</td>
<td>(13-42)</td>
<td>&lt;0.0008</td>
<td>0.90</td>
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Table 2: Relative risk reductions associated with a 10% lower mean HbA\textsubscript{1c} among HbA\textsubscript{1c} values ≤8 vs. values >8% estimated from a segmented (change point) model.

Glycemia and Complications in the DCCT

Ropathy, demonstrated similar associations with HbA\textsubscript{1c} level within the HbA\textsubscript{1c} ≤8% range and the >8% range. The risk reductions ranged from 21 to 49% for a 10% lower mean HbA\textsubscript{1c} and were similar for the intensive and conventional treatment groups. In all instances, there was a further reduction in risk with reductions in HbA\textsubscript{1c} <8%, and in most cases, this additional reduction itself was statistically significant. However, in no case was there a significant difference between the risk gradients evaluated for HbA\textsubscript{1c} ≤8% and for HbA\textsubscript{1c} >8%, thus failing to establish a significant change point at an HbA\textsubscript{1c} of 8% and rejecting the hypothesis of a threshold at an HbA\textsubscript{1c} of 8%. In no instance did the two-segment (change point) regression model provide a significantly better fit to the data than did the simple exponential regression model presented in Table 1 and in Figs. 2 and 3.

In further analyses of retinopathy progression using proportional hazards models, stratified-adjusted for treatment group and baseline retinopathy severity, there was again no difference in the risk gradients for HbA\textsubscript{1c} ≤8% and >8%. In analyses of the sustained onset of retinopathy in the primary cohort and of the development of SNPDR among patients in the secondary cohort (Table 2), there were no differences between the risk gradients for ≤8% vs. >8% in either treatment group or in the groups combined. These analyses again indicate a significant continuing relative reduction in risk of sustained retinopathy progression among intensive group patients with HbA\textsubscript{1c} ≤8%. The relative risk reduction was not significantly less than that observed among values >8%.

In general, PH models of retinopathic and nephropathic outcomes yielded the same results as those presented in Table 2 from the simpler Poisson models. In no instance did the segmented model provide a significant improvement over the simple exponential model. We searched for evidence of a significant change point or a threshold using PH models for microalbuminuria and sustained retinopathy over the range of HbA\textsubscript{1c} from 6.0 to 9.9% in increments of 0.1%, and found none.

Hypoglycemia. The current HbA\textsubscript{1c} value, measured monthly in the intensive treatment group and quarterly in the conventional treatment group, was more strongly associated with the risk (incidence) of all episodes of hypoglycemia (including recurrent episodes) than was the updated mean HbA\textsubscript{1c}. Among patients in the intensive and conventional groups, analysis of the risk gradients for the incidence of all severe hypoglycemia and for episodes with coma/seizure, expressed as the percentage increase in risk per 10% lower current HbA\textsubscript{1c}, revealed that the risk gradients were significantly higher in the conventional than in the intensive group. For each 10% reduction in the HbA\textsubscript{1c}, there was a 26% increase in the risk of all severe hypoglycemia (95% CI 22-29%) in the intensive treatment group versus a 54% increase in risk (95% CI 49-60%) in the conventional group (P < 0.0001 for intensive versus conventional). For hypoglycemia with coma/seizure, the risk gradients were likewise markedly different between the intensive and conventional treatment groups (18 vs. 48% increase in risk, respectively, per 10% reduction in HbA\textsubscript{1c}; P < 0.0001). Thus, we did not combine the treatment groups for the examination of a change point.

For the intensive and conventional treatment groups, the relative risk gradients associated with a 10% reduction in HbA\textsubscript{1c}, obtained from segmented regression models with a change point at an HbA\textsubscript{1c} of 8% are presented in Table 3. For episodes of severe hypoglycemia in the intensive treatment group, the risk gradient among HbA\textsubscript{1c} values ≤8% (18% greater risk per 10% lower HbA\textsubscript{1c}) and that among values >8% (60% greater risk per 10% lower HbA\textsubscript{1c}) were each significant at P < 0.0001. Furthermore, the risk gradient for hypoglycemia for HbA\textsubscript{1c} values ≤8% was significantly less than that for values >8% (18 vs. 60%, P < 0.0001). Similar results were observed in the conventional treatment group, in which the risk gradients over these two segments were also significantly different (43 vs. 72%, P = 0.005).

For episodes with coma/seizure in the intensive group, the risk gradient among HbA\textsubscript{1c} values ≤8% (7% greater...
risk per 10% lower HbA1c) was again significantly lower (P = 0.003) than that among values >8% 61% greater risk per 10% lower HbA1c). Moreover, among HbA1c values ≤8%, this risk gradient in the intensive group was low and was nominally not significantly different from zero (P = 0.062), although this may in part be a function of the smaller number of such episodes than all episodes of severe hypoglycemia. In the conventional group, the risk gradients over these two segments were not significantly different (44 vs. 52%, P = 0.64).

These analyses indicate that in the intensive treatment group, the risk of severe hypoglycemia increased more slowly with reductions in HbA1c ≤8%, compared to the rate at which the risk increased for reductions in HbA1c in the range of >8%. In contrast to the results with long-term complications, a two-parameter segmented model provided a significantly better description of the trend in the risk of hypoglycemia as a function of the HbA1c than the simple exponential curve previously presented (Fig. 5B in Ref. 2).

**DISCUSSION**

The DCCT, with 9,065 patient-years of prospective follow-up and 4,835 patient-years with a mean HbA1c ≤8% and using precise and frequent measures of glycemia and complications over time, provides an excellent opportunity to assess the relationships between glycemic exposure and the risk of complications. We have demonstrated previously that the dominant predictor of the risk of retinopathy is the total lifetime exposure to hyperglycemia as reflected by the level of HbA1c and the duration of diabetes (4).

Herein we show that there is a linear relationship between the log of the absolute risk (instantaneous hazard rate) of retinopathy and nephropathy and the log of the current HbA1c over the entire range of HbA1c values (Figs. 2A and 3A). This equates to a nonlinear relationship between the untransformed risk of complications and the untransformed level of HbA1c, a relationship that is based on a constant relative risk in both the intensive and the conventional treatment groups (Figs. 2B and 3B). For a proportionate reduction in HbA1c there is a constant proportionate reduction in risk.

This nonlinear relationship means that the absolute reduction in risk is greatest when high values of HbA1c are reduced. A reduction in HbA1c from 11 to 9.9% yields a reduction in risk of retinopathy progression of 6.57 cases per 100 patient-years (10.78 - 4.21). In contrast, a reduction from 8 to 7.2% yields a reduction in risk of 0.95 cases per 100 patient-years (2.43 - 1.48). Although each represents a 10% reduction in HbA1c, and each results in the same proportionate (39%) reduction in the risk of retinopathy progression, the magnitude of the reduction in absolute risks is much greater in the former than in the latter case. All of these associations use the current HbA1c value; however, if we use the mean change from baseline to current HbA1c in the model, we obtain virtually identical risk reductions in retinopathy progression for 10% lower HbA1c.

The recent suggestion that decreasing HbA1c below 8% is not accompanied by further reduction in risk (6,8) (the threshold hypothesis) is refuted by these analyses of the extensive DCCT data. Moreover, our analyses of the risks of retinopathy, nephropathy, and neuropathy within the intensive, conventional, and combined groups in the DCCT revealed no evidence that an HbA1c of 8% or any other value represented a significant threshold or change point in the relative risk relationship (Table 2). In most instances, the relative risk gradient over the segment of HbA1c values ≤8% was significantly different from zero, rejecting the hypothesis that a threshold exists at an HbA1c of 8%. In all instances, the overall relative risk gradient was significant and no difference was found between the risk gradients over the two segments of HbA1c ≤8% versus values >8%, demonstrating no discernible change point at an HbA1c of 8%. Thus, for each complication, the DCCT data indicate a continuous relative risk relationship over the range of HbA1c values, with no apparent glycemic threshold or change point.

Furthermore, the fact that the intrinsic risk relationship between levels of glycemia and complications is nonlinear implies that two straight lines could be fit to the data in Figs. 2B or 3B (without the log transformation), with different slopes over the range of HbA1c values ≤8% and for values >8%, as was done by Krolewski et al. (6). However, such an analysis obscures the fact that there is a constant relative risk relationship over the entire range of HbA1c values.

Several other studies, including a large (n = 3,250) cross-sectional study of IDDM and its complications (21), the prospective Wisconsin Epidemiologic Study of
Diabetic Retinopathy (22,23), the prospective Stockholm Diabetes Intervention Study (24), and others in Colorado (25) and Berlin (26), have reported relationships between HbA₁c and various complications that agree with our findings and do not support the existence of glycemic thresholds for the early stages of retinopathy, nephropathy, and neuropathy studied in the DCCT. However, some of these studies have suggested the possibility of a glycemic threshold for the development of proliferative retinopathy (21,24). There were too few such events during the DCCT for our data to address this possibility directly. Analyses of severe nonproliferative diabetic retinopathy, the stage a few steps short of proliferative retinopathy on the ETDRS scale (13), failed to demonstrate any glycemic threshold in the DCCT (Table 2).

Features of the Krolewski et al. (6) study likely contributed to their failure to demonstrate evidence of risk reductions in microalbuminuria at HbA₁c values ≤8%. One important statistical factor is the influence of random variation or noise. Only one to three random urine albumin-to-creatinine ratios were measured. It is well known that levels of albumin in the microalbuminuric range are highly variable over time within patients, whereas albuminuria, once developed, is more stable. Further, the patients who had reached clinical albuminuria (proteinuria) were eliminated from the Krolewski et al. (6) analysis. Thus, they attempted to correlate a noisy outcome (microalbuminuria measured in spot urine specimens) with HbA₁c, weakening the sensitivity of their analysis and excluding the patients of greatest clinical interest (those with albuminuria).

The DCCT and some of the studies cited above (22–24,26) were able to describe a risk relationship with microalbuminuria (and other complications) because periodic longitudinal measurements in large numbers of patients can override the influence of random within-patient variation over time. Also, in the DCCT, the slope of the risk relationship between glycaemia (HbA₁c) and microalbuminuria (Fig. 3A) is not as large as that for sustained retinopathy (Fig. 24). This suggests that retinopathy may be more sensitive to the effects of glycaemia than is microalbuminuria, as measured in the DCCT.

The matter of greatest clinical interest is the magnitude of further reductions in risk of complications with HbA₁c values ≤8%. For example, from the regression model in Table 1 and Fig. 2B, the estimated risk of retinopathy progression is 2.43 cases per 100 patient-years at an HbA₁c of 8%, which is reduced to 1.30 at an HbA₁c of 7% and to 0.63 at an HbA₁c of 6%. However, each of these rates per 100 patient-years is in fact an instantaneous risk (hazard rate), which can be interpreted (approximately) as the percentage of patients among those still at risk who will first experience the event in a year's time. When compounded over a 9-year period of follow-up as in the DCCT (Fig. 2D), these rates correspond to 25% cumulative incidence of patients developing retinopathy progression when held at an HbA₁c of 8%, versus 11% at an HbA₁c of 7%, and only 5.5% at an HbA₁c of 6%.

Likewise, from Fig. 3B, the estimated risk of microalbuminuria is 3.41 cases per 100 patient-years at an HbA₁c of 8%; 2.35 at an HbA₁c of 7%; and 1.53 at an HbA₁c of 6%. When compounded over a 9-year period of follow-up (Fig. 3C), these hazard rates correspond to 25% cumulative incidence of patients developing microalbuminuria when held at an HbA₁c of 8% over 9 years, versus 19% at an HbA₁c of 7%, and 13% at an HbA₁c of 6%.

When projected over longer periods of treatment, these differences in the cumulative incidence of retinopathy progression or microalbuminuria diverge exponentially for different values of HbA₁c. Therefore, when the absolute instantaneous risks associated with HbA₁c values ≤8%, albeit progressively smaller, are applied to hundreds of thousands of patients and are compounded year by year over a lifetime of diabetes, there are substantial differences in the numbers of patients affected at the different HbA₁c levels over the range of 6 to 8%.

It is also important to note that 83% of intensively treated subjects in the DCCT achieved a mean HbA₁c ≤8% during the trial, the median value among all subjects being 7.07%. When compared with conventional treatment with a median of 9.02%, this reduction in HbA₁c with intensive therapy led to dramatic reductions in the risks of complications, including a 70% reduction in the risk of sustained retinopathy progression and a 39% reduction in the risk of microalbuminuria. However, from Figs. 2D and 3B, it is estimated that an intensive treatment with an average HbA₁c of 8%, compared with conventional treatment, would achieve only a 43% reduction in the risk of sustained retinopathy progression and a 28% reduction in the risk of microalbuminuria.

It could be argued that the absolute further gains in preventing complications by lowering HbA₁c below 8% would not be worth the effort, risk, and cost required. For example, treatment of retinopathy with photocoagulation at the earliest appropriate time does offer a practical and relatively low-risk treatment (27) for the large residue of retinopathy that would accrue if all patients with IDDM were treated by only seeking an HbA₁c goal of 8%, as recently suggested (5–8). However, many patients do not reach the ophthalmologist in time (28) or have the severity of their retinopathy recognized (29). In addition, photocoagulation both is costly and can itself cause loss of vision (27). Similar conclusions apply to neuropathy, which all too often leads to foot ulcers and amputation (30) because preventive measures are frequently ignored in practice (31). Only intensive therapy offers the chance of maximally decreasing the risk of development and progression of all of the diabetes-specific complications.

If an HbA₁c of 8% were adopted as a standard, the greatest concern would be the thousands of patients who might develop renal failure and be encumbered with the morbidity, mortality, and expense associated with end-stage renal disease and its treatment (32). There is ample evidence that elevated albumin excretion presages ultimate renal insufficiency (33,34). The DCCT has demonstrated maximal reduction in the risk of microalbuminuria and albuminuria when therapy is aimed at lowering the HbA₁c as much as possible. Decreasing the development and progression of the earlier stages of nephropathy is very likely to decrease and/or delay the development of end-stage renal disease. Given the catastrophic
nature of end-stage renal disease to the patient and the huge cost to society, it is imperative that physicians recommend the most effective current therapy aimed at the lowest HbA1c, that can be safely achieved. From a practical point of view, aiming for an HbA1c goal of 8% is likely to result in values that are considerably higher. In the DCCT, the actual mean HbA1c achieved was ~1% higher than the goal of <6.05%.

A major incentive for other authors to select HbA1c goals higher than the target used in the DCCT was the belief that higher HbA1c goals would substantially lower the risk for severe hypoglycemia (5–8). However, our data reveal, in contrast to the relationship between HbA1c and the risk of developing complications, that the risk of severe hypoglycemia does not increase proportionally as HbA1c is reduced over the range of values ≤8% as it does over the range of values >8%. Although the risk of severe hypoglycemia continues to increase at lower HbA1c values with intensive therapy, the risk gradient flattens substantially. Therefore, as the HbA1c levels are reduced below 8% with intensive therapy, there is a continuing proportionate reduction in the risk of complications, whereas the risk of severe hypoglycemia increases at a slower rate. The mechanism for this flattening of the risk curve for hypoglycemia is unknown. However, it may be that the risk for all hypoglycemia (which includes recurrent episodes) tends to flatten because patients who experience severe reactions are more likely to practice preventive behavior that ameliorates their risk for hypoglycemia without substantially affecting their HbA1c.

Given the demonstrated association between glycemia and HbA1c in both the conventional and intensive treatment groups, it is tempting to advocate any therapy that achieves the target HbA1c of intensive therapy. However, since intensive therapy as implemented in the DCCT and other controlled clinical trials (24) is the only therapy that has been tested directly and demonstrated both to lower glycemic levels and to prevent or delay long-term complications, the DCCT Research Group continues to recommend it as the first choice for most patients with IDDM. Until other therapies are proven equally effective and at least as safe as intensive therapy, intensive therapy should remain the treatment of choice for IDDM.

In conclusion, the recent assertion that a glycemic threshold exists at an HbA1c of 8% (6,8) is not supported by the best available data. Further, the therapeutic recommendation that the target level of HbA1c be set at 8% could well do harm. Therefore, the DCCT continues to recommend implementation of intensive therapy with the goal of achieving normal glycemia as early as possible in as many IDDM patients as is safely possible.

ACKNOWLEDGMENTS
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The role of T-cells in the pathogenesis of IDDM has been an area of much interest, and investigators have recently acquired new tools for studies on T-cells with the advent of T-cell clones that are reactive with islet antigens. Derived from NOD mice, diabetogenic T-cell lines and clones have for the most part been CD4⁺ and T-helper 1 (Th1)-like in their cytokine production. Some CD8⁺ cytotoxic clones have also been reported, although these have generally not transferred diabetes in the absence of CD4⁺ T-cells. The T-cell clones that have been described can also be separated on the basis of their antigen reactivity. While many of the T-cell lines and clones described react with islets, isolated islet cells, or islet membrane preparations, others have known antigen specificities, reacting with defined islet cell proteins such as insulin, GAD, and heat shock proteins. Particularly in the case of insulin-reactive clones, diabetogenicity has also been demonstrated. In light of the many possible T-cell reactivities that may arise from the islet lesion, the question of whether there is a dominant initiating antigen is a particularly intriguing one. Diabetes 45:1299-1305, 1996

The two most distinctive features of type I diabetes, or IDDM, as an autoimmune disease are the presence of autoantibodies in the sera of patients and the mononuclear cellular infiltration of islets known as insulitis. From about the mid-70s to the mid-80s, much attention was focused on the humoral aspects of autoimmunity as researchers tried to define β-cell autoantigens through the isolation and characterization of autoantibodies arising spontaneously in sera of patients and animals with IDDM or as a result of immunization with various islet and pancreatic tissue preparations. This approach succeeded to a large extent in that a number of important autoantibody reactivities were identified, including those to insulin, GAD, carboxypeptidase H, ICA69 (islet-cell antigen 69), and several gangliosides (1). Perhaps one of the biggest dividends of this work is the basis that has been laid for diagnosing at-risk populations through analysis of their autoantibody titers; as more reliable assays are developed for defined β-cell autoantigens such as insulin, GAD, and ICA69, mass screening of potential diabetic patients will become more feasible (2). However, no direct role for autoantibodies in the pathogenesis of diabetes has been established.

During the mid-80s, increasing interest focused on cell-mediated autoimmunity and the nature of the infiltrate in the islets, especially on the role of T-lymphocytes (or T-cells). Histological analysis of islet lesions revealed that T-cells were a predominant component of the infiltrate and that both CD4⁺ and CD8⁺ T-cells were present (3). A good deal of work, summarized in several recent reviews (4-6), has demonstrated the importance of T-cells in the pathogenesis of diabetes: disease was prevented by treatments that included neonatal thymectomy, antibodies to T-cell molecules, and immunosuppressant drugs that affect T-cells, and most convincingly, disease could be adoptively transferred with splenocytes from diabetic mice.

Early adoptive-transfer experiments, in which fractionated spleen cells were transferred into neonatal or sublethally irradiated adult recipients, indicated that both CD4⁺ and CD8⁺ T-cells were required to induce diabetes (7,8), but the relative importance of each subset has been more difficult to assess. In general, CD4⁺ T-cells seem to be primarily involved in the initiation of the disease process and in recruiting other cells (including CD8⁺ T-cells) to the pancreas. The role of CD8⁺ T-cells in β-cell destruction has been more controversial. The recent development of the NOD-scid mouse has allowed a more detailed analysis of the role of CD4⁺ and CD8⁺ T-cells in which it has been observed that the degree of CD8 dependence of disease transfer was donor-age dependent (9).

Thus, when spleen cells from overtly diabetic donors were depleted of CD8⁺ T-cells, there was decreased diabetes incidence and delayed time to onset; however, when 6- to 8-week-old donors were used, there was an absolute dependence on both CD4⁺ and CD8⁺ T-cells. These results suggest that CD8 T-cells are required for development of a pathogenic T-cell response to islet cells, but once a CD4 response develops, CD8⁺ T-cells are not absolutely necessary.

With the advent of cloned T-cell lines, we now have at hand the tools to begin dissecting the individual contributions of different T-cell subsets and specificities. For
instance, information on the relative contributions of CD4+ and CD8+ T-cells to β-cell destruction, as well as interactions between these two populations, could be provided by analysis of representative examples of each of these types of cells in the form of islet-specific T-cell clones. In this review, we describe T-cell clones that have recently become available, with particular emphasis on those that are diabetogenic. Although there have been numerous reports of T-cell lines and clones reacting with islets and islet-derived antigens, very few of these T-cells have been well characterized with respect to pathological activity. This becomes an important issue when one considers the relevance of human T-cell clones and whether they are pathogenic.

**ISLET-REACTIVE T-CELL CLONES**

Barbara Davis Center for Childhood Diabetes (BDC) panel of islet-specific diabetogenic T-cell clones. Although there have been a few reports of islet-reactive T-cell lines isolated from human peripheral blood lymphocytes (10,11) or from the BB rat (12), nearly all of the cloned T-cell lines described within the last 10 years have come from the NOD mouse. The first report of a pathogenic T-cell clone came from the Barbara Davis Center for Childhood Diabetes (BDC) and was described by Haskins et al. (13). A CD4+ T-cell clone, BDC-2.5, was derived from a newly diabetic NOD mouse and cultured in the presence of mouse islet cells and irradiated splenocytes as antigen-presenting cells (APCs). When assayed with islet cells and NOD APCs, this clone proliferated and made interleukin (IL)-2; it did not respond to either islet cells or APCs alone, which suggests that it recognized an islet cell antigen in the context of the NOD class II molecule, I-A^k. In vivo, the BDC-2.5 clone was indicated to be pathogenic by virtue of the fact that it mediated islet but not pituitary graft destruction. A second clone, BDC-2.4, did not display specificity for islet cells in vitro and did not affect islet grafts. Haskins et al. (14) subsequently produced a panel of cloned T-cell lines, all CD4+ and derived from spleen and lymph node cells of newly diabetic female NOD mice, that responded in vitro to islet cells in the presence of NOD APCs by proliferating and making IL-2. Further analysis indicated that these clones were all of the T-helper 1 (Th1) phenotype in that they produce the cytokines IL-2, γ-interferon (IFN-γ), and tumor necrosis factor (TNF), but not IL-4 (15), and that their T-cell receptor variable and joining regions were heterogeneous (16). In vivo, it was found that every islet-specific clone that was tested could mediate islet graft destruction in (CBA × NOD)F1 recipients, animals that are not prone to developing spontaneous diabetes but are rendered diabetic with streptozotocin and then transplanted with NOD islets (14,17). The diabetogenic nature of these clones was confirmed by studies in which two or three injections of two of the T-cell clones, BDC-2.5 and BDC-6.9, were administered intraperitoneally to young NOD recipients; mice receiving these two clones (but not controls receiving the non-islet-specific clone BDC-2.4) rapidly developed diabetes and/or extensive insulitis (18).

Over the last few years, extensive studies have been carried out to characterize the in vivo activity of this panel of islet-specific T-cell clones. In the first studies, in which the T-cell clones were adoptively transferred into very young (2- to 3-week-old) NOD recipients (18), we observed that efficiency of disease induction appeared to correlate with the age of the mice. Extensive insulitis developed in all of the animals that received islet-specific T-cell clones, but as mice approached 3 weeks of age, the incidence of overt diabetes, indicated by hyperglycemia, decreased. Further investigation of the kinetics of disease induction revealed that in unirradiated NOD mice, there is a very narrow age window in which disease can be efficiently transferred with T-cell clones (19). In the three age-groups studied, it was found that four different diabetogenic T-cell clones caused disease with high efficiency (75% incidence of overt diabetes with three of four clones, 67% overall) in the youngest (8- to 14-day-old) group, with lower efficiency in the 15- to 18-day-old group, and not at all in the third age-group (19- to 28-day-old). These results, together with similar findings by Bendelac et al. (7), who investigated disease induction with diabetic splenocytes in three age-groups of NOD recipients, suggested that there is a developmental event in the NOD mouse after 3 weeks of age that confers resistance to T-cell transfer of disease.

In another set of studies, the question was whether the T-cell clones could adoptively transfer diabetes to animals not prone to developing spontaneous disease, e.g., F1 crosses between NOD mice and other strains. Four diabetogenic clones were used in transfer experiments in seven different NOD F1 strain combinations, including three in which there was positive expression of the class II I-E. Our results showed that although there was variability among different clones in different F1 recipients, all four clones were able to transfer diabetes to three or more F1 combinations (20). However, there was one strain combination, the (C57L × NOD)F1, that was highly resistant to disease transfer, an interesting observation in light of work by McDuffie and coworkers describing three diabetes-resistant NOD congenic lines that were produced from a C57L × NOD cross and the evidence that three separate and independent genes are involved in conferring resistance in these lines (M. McDuffie, Abstract, IXth International Congress of Immunology, July 1995). Furthermore, disease transfer studies in the NOD congenic mice with the diabetogenic T-cell clone BDC-6.9, indicated that clone-induced diabetes was delayed in two of the mouse lines compared with NOD controls (100% of controls became diabetic with BDC-6.9 <10 days after the first injection of cells). In the third line, however, there was a significant degree of protection: in 100% of the mice that received BDC-6.9, no overt diabetes was observed in the 14-day period before the animals were killed (M. McDuffie and K.H., unpublished observations).

We have also conducted studies using islet-specific T-cell clones to investigate the relative roles of CD4+ and CD8+ T-cells in the induction of diabetes. In a continued collaborative effort with Lafferty and coworkers (21,22) to investigate the ability of the clones to mediate islet graft destruction, it was found that the T-cell clone BDC-6.9 could migrate to the graft site when injected intraperitoneally into (CBA × NOD)F1 mice that had received NOD islet transplants and that islet destruction took
place whether or not CD8+ T-cells were present. This work, along with earlier descriptions of diabetes transfer with the T-cell clones in young NOD mice (18), suggested that at least in the case of an activated T-cell clone, CD4+ T-cells were sufficient for disease induction. More recently, a better model has become available for exploring the individual contributions of different T-cell subsets: the NOD/Lt-scid/scid (NOD-scid) mouse described by Christianson et al. (9). The NOD-scid mouse, like other scid mice, does not have T- or B-cells and is not prone to diabetes. However, adoptive transfer of splenocytes from diabetic NOD mice to NOD-scid mice will reproducibly result in full-blown diabetes, characterized by hyperglycemia and severe insulitis, within a 3- to 4-week period (9,15). Using this immunodeficient mouse as a recipient, we demonstrated that transfer of the islet-specific CD4+ T-cell clone BDC-6.9 caused destruction of pancreatic β-cells and development of diabetes without help from host B-cells, CD4+ T-cells, or CD8+ T-cells (15). However, a second islet-specific T-cell clone, BDC-2.5, could not transfer diabetes or insulitis into NOD-scid mice. Although it readily induces diabetes in young unmanipulated NOD mice, BDC-2.5 could only induce disease in NOD-scid mice with a cotransfer of CD8-enriched T-cells from diabetic spleen. In young NOD mice receiving the VB4+ T-cell clones BDC-2.5 or BDC-6.9, immunohistochemical staining of pancreatic lesions showed the presence of CD4+, CD8+, VB4+, and MAC-1+ cells within the infiltrate, as in the infiltrates in lesions of spontaneously diabetic female NOD mice. In NOD-scid mice that received BDC-6.9, there were CD4+VB4+ T-cells and a large population of MAC-1+ cells in islet lesions. In NOD-scid recipients of cotransferred BDC-2.5/CD8+ splenic T-cells, there was a small population of CD4+ T-cells and a larger population of CD8+ T-cells in the islets, whereas no infiltrate was detectable in recipients of CD8+ splenocytes or BDC-2.5 alone. These results indicate that there may be a role for CD8+ T-cell help in diabetes induced by some islet-specific CD4+ T-cell clones.

**Other islet-reactive T-cell clones.** That there is considerable diversity in the types and activities of T-cell lines and clones produced from NOD mice is indicated by reports from other investigators. Unlike the BDC clones, which were derived from spleen and lymph node T-cells and are all CD4+, almost all other T-cell lines that have been described were isolated from the islet lesions of NOD mice and included CD8+ as well as CD4+ T-cells. For example, Reich et al. (23) described two cloned T-cell lines derived from NOD islets, one CD4+ and the other CD8+, that responded to NOD islet cells and that could in combination (but not singly) induce extensive insulitis in 7- to 8-week-old sublethally irradiated NOD or (NOD × BALB/c)F1 recipients (23). Another report from Nagata et al. (24) described the isolation of cytotoxic CD8 T-cell lines by culturing islets from 20-week-old female NOD mice in IL-2; these cells were capable of specific killing of islet cells, but no analysis was made of their activity in vivo.

Nakano et al. (25) isolated from islet infiltrates of NOD mice CD4+ T-cell clones that responded to islets from various strains of mice in the context of NOD APCs, showed diverse usage of T-cell receptor V and J segments, and transferred insulitis into I-E+ transgenic NOD mice (25). In a report by Panekwycz et al. (26), T-cell lines were propagated from pancreatic islets of prediabetic (2-month-old) NOD mice and yielded a CD4+ clone that responded to islet cells and induced disease in female NOD mice. Shimizu et al. (27) isolated CD4+ and CD8+ T-cell clones from islets obtained from irradiated nondiabetic male NOD mice that had received a transfer of splenocytes from diabetic females. These clones proliferated and made IFN-γ in response to islet cells in the presence of NOD APCs, and the CD8 clones also killed islet cells. Two of the CD4+ T-cell clones were diabeticogenic in irradiated NOD male recipients, particularly in the presence of splenic CD8+ T-cells from diabetic mice.

In a report on the isolation of CD4+ and CD8+ T-cell clones from islets of diabetic female NOD mice, Hagata and Yoon (28) found that CD4+, but not CD8+, T-cell lines could be isolated from animals <10 weeks of age and concluded that CD4+ T-cells may be involved primarily in the initiation of disease, whereas CD8+ T-cells are late-stage effectors. A later study by this group indicated that the CD8+ CTL lines could induce disease in 7- to 8-week-old sublethally irradiated NOD recipients, but only in the presence of CD4+ T-cells (29). To isolate CD4+ and CD8+ T-cell lines from islet infiltrates, Maugendre et al. (30) used an interesting approach in which culture wells were precoated with antibodies to T-cell receptor VB6 or VB8; lines with both phenotypes were found to be cytotopic with CD3-coupled P815 targets or yeast artificial chromosome (YAC) cells, but islet cell specificity was not determined. One VB6/CD4 line could induce insulitis in neonatal recipients, but only when injected together with CD8+ T-cells. Another novel approach to deriving islet-specific T-cell lines was used by Wegmann et al. (31), who used syngeneic islet grafts in NOD recipients to recruit autoreactive T-cells. Several lines with islet specificity were obtained, and clones from one line, N1, were shown to be diabeticogenic in NOD-scid recipients. Gelber et al. (32) reported on the isolation of lines and clones that were reactive with various fractions produced from extracts of a β-cell insulinoma and that were in some cases diabeticogenic. Most recently, Healey et al. (33) described CD4+ T-cell lines that were reactive with extracts from a rat insulinoma and that included both Th1 (IFN-γ-secreting) T-cells and Th2 (IL-4-secreting) T-cells. The Th1 lines could induce insulitis and diabetes, whereas injections with the Th2 line resulted in only a mild peri-islet infiltrate (33).

The diabeticogenicity of these various CD4+ and CD8+ T-cell lines and clones is apparently quite variable from group to group. In some reports, the pathogenesis described was in the form of insulitis, not overt diabetes (23,25). In several studies, including our own, CD4+ T-cell clones alone induced disease (18,25,27,31), but in others, only a combination of CD4+ and CD8+ clones resulted in pathogenesis (23,29,30). In the case of islet-reactive CD8+ T-cells, investigators generally have not observed disease transfer when CD8+ clones are used alone, but only when they are cotransferred with CD4+ lines or spleen cells. However, Wong et al. (34) have recently reported on CD8+ T-cell clones isolated from 7-week-old female NOD mice and cultured on islets expressing the B7-1 costimulatory
molecule. These CD8+ T-cell lines and clones were H-2K\(^d\)-restricted and were found to proliferate and be cytotoxic to NOD islets in vitro. In adoptive-transfer experiments to NOD- or CB17-scid recipients, diabetes developed rapidly and in the absence of CD4+ T-cells.

**Antigen specificity of islet-reactive clones.** All of the T-cell clones described above (and summarized in Table 1) are termed islet-reactive because they respond to islets or islet cells in T-cell assays in vitro. Purification of unknown antigens from islet tissue is not a simple task, and in most cases, little is known about the antigen targets for these T-cells. Several labs have reported CD4+ NOD T-cell lines or clones that react to islet cells from multiple mouse strains in a NOD class II-restricted manner (14,25,27), whereas CD8+ T-cells respond to islet cells alone (23,27). The work of Shimizu et al. (27) indicated that the antigen for their CD4+ T-cell clones could not be detected in conditioned media from islet cells, but live APCs with formaldehyde-fixed islet cells did stimulate responses, suggesting that a membrane-associated protein was responsible for antigenicity.

Evidence for an undefined membrane-associated antigen was further provided by studies with the BDC panel of CD4+ islet-specific T-cell clones. From an extensive analysis of the subcellular location of islet antigens for these clones, Bergman and Haskins (35) found that a fraction that was highly enriched in β-granule granules was antigenic for every islet-specific T-cell clone tested from the panel, and we subsequently localized that activity to the β-granule membrane. More recent work has indicated that the antigenic activity for at least two T-cell clones can be isolated by anion exchange chromatography of detergent-solubilized β-granule membranes and further purified by size-exclusion chromatography, yielding an active fraction that falls into a molecular weight range of 50–80 kDa (B. Bergman, J.L. McManaman, K.H., unpublished observations).

Because of the unique islet cell response of the T-cell clone BDC-6.9, Haskins and colleagues were also able to take a genetic-mapping approach to identifying the antigen for one of the BDC clones (36). It was found that BDC-6.9 reacts only to islet cells from the NOD mouse, from its related strains NOR and NON mice, and from SWR mice, whereas most of the BDC clones, including BDC-2.5, react to islet cells from all of the inbred mouse strains tested. We produced an (NOD × BALB/c) × BALB/c backcross and looked for correlations between chromosomal loci mapped by microsatellite markers and the presence or absence of antigenicity in islets from each backcross mouse. The result was that a locus in the telomeric region of mouse chromosome 6 was found to have a 100% correlation with the presence of the islet antigen in the backcross (36).

**T-CELL CLONES REACTIVE TO DEFINED ISLET ANTIGENS**

**Insulin-specific T-cell clones.** As more information has become available with regard to β-cell autoantigens defined by autoantibody reactivity, investigators in recent years have begun to ask whether these autoantigens are also T-cell targets. Because insulin comprises ~80% of the β-cell protein, as well as being critical in prevention and control of diabetes, its role as an autoantigen is of great interest. The first report of insulin-specific T-cell lines came from Wegmann et al. (37), who described CD4+ T-cell lines and clones isolated from the islet infiltrates of unimmunized 7- and 12-week-old NOD mice. Of T-cell clones isolated from the older mice, about half were found to react with insulin; insulin reactivity was a result of in vitro selection.

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**Table 1**

<table>
<thead>
<tr>
<th>Origin of T-cells</th>
<th>CD4/CD8</th>
<th>Antigen specificity</th>
<th>In vivo activity</th>
<th>Investigators</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spleen and lymph nodes of diabetic females</td>
<td>CD4</td>
<td>Islet cells, β-granule membrane</td>
<td>Insulitis and diabetes</td>
<td>Haskins et al. (13-22), Bergman and Haskins (35)</td>
</tr>
<tr>
<td>Islet infiltrates of diabetic females</td>
<td>CD4, CD8</td>
<td>Islet cells</td>
<td>Insulitis</td>
<td>Reich et al. (29)</td>
</tr>
<tr>
<td>Islet infiltrates of 20-week-old non diabetic females</td>
<td>CD8</td>
<td>Islet cells</td>
<td>ND</td>
<td>Nagata et al. (24)</td>
</tr>
<tr>
<td>Islet infiltrates of 7- to 11-week-old non diabetic mice</td>
<td>CD4</td>
<td>Islet cells</td>
<td>Insulitis</td>
<td>Nakano et al. (25)</td>
</tr>
<tr>
<td>Islet infiltrates of 8-week-old non diabetic females</td>
<td>CD4</td>
<td>Islet cells</td>
<td>Insulitis and diabetes</td>
<td>Pankewycz et al. (26)</td>
</tr>
<tr>
<td>Infiltrated islets of an irradiated nude recipient</td>
<td>CD4, CD8</td>
<td>Islet cells</td>
<td>Diabetes (CD4 clones only)</td>
<td>Shimizu et al. (27)</td>
</tr>
<tr>
<td>Islet infiltrates of &lt;10- and &gt;10-week-old non diabetic females</td>
<td>CD4, CD8</td>
<td>Islet cells</td>
<td>Diabetes (CD4 with cotransfer of CD4 T-cells)</td>
<td>Nagata and Yoon (28)</td>
</tr>
<tr>
<td>Infiltrated islet isografts</td>
<td>CD4</td>
<td>Islet cells</td>
<td>Insulitis and diabetes</td>
<td>Wegmann et al. (31)</td>
</tr>
<tr>
<td>Spleen and lymph nodes of 30- to 40-day-old non diabetic females</td>
<td>CD4</td>
<td>Insulinoma extracts</td>
<td>Insulitis</td>
<td>Gelber et al. (32)</td>
</tr>
<tr>
<td>Lymph nodes of diabetic females</td>
<td>CD4</td>
<td>Islet cells</td>
<td>Diabetes and insulin</td>
<td>Healey et al. (33)</td>
</tr>
<tr>
<td>Islet infiltrates of 7-week-old non diabetic females</td>
<td>CD8</td>
<td>Islet cells</td>
<td>Diabetes</td>
<td>Wong et al. (34)</td>
</tr>
</tbody>
</table>
Further characterization of the insulin-specific T-cell clones was provided in a later report in which the functional properties and epitope specificity of the clones were investigated (38). It was found that six clones derived from 12-week-old NOD mice, five were Th1-like in that they secreted IFN-γ and no IL-4; one clone, however, secreted both cytokines. To varying degrees, all of these T-cell clones showed diabetogenic potential in young (<14-day-old) NOD recipients, causing diabetes and/or extensive insulitis. One clone was also able to transfer diabetes into the lymphocyte-deficient NOD-scid mouse. To determine which insulin epitopes were antigenic for the insulin-specific T-cell clones, overlapping 15-amino acid synthetic peptides from both isoforms of mouse insulin were tested, and interestingly, all of the six clones and a number of uncloned insulin-reactive T-cell lines responded only to the peptide encompassing residues 9-23 of the insulin B chain. This restricted antigen specificity for peptide B-(9-23) is different from insulin-specific T-cells derived from non-diabetes-prone mouse strains immunized with insulin and reactive to either the insulin A chain or a combinatorial A chain–B chain determinant; it may be that the B-(9-23) peptide is unique in terms of being autoantigenic. The importance of the B-(9-23) peptide has been further confirmed by a recent study by Daniel and Wegmann (39) in which it was shown that this was the dominant epitope for >300 insulin-specific T-cell clones and that intranasal and subcutaneous administration of this peptide resulted in significant delay in onset and decrease in incidence of diabetes compared to that in mice given a control peptide from tetanus toxin.

CD4+ insulin-specific T-cell lines have also been successfully produced from NOD mice that were immunized with various forms of insulin, including bovine, ovine, and porcine insulin as well as A or B chains from bovine insulin, and both A and B chain determinants were found to be antigenic (A. Cooke, unpublished observations). The unique autoantigenic nature of the insulin B-(9-23) peptide was again demonstrated by the isolation of a diabetogenic CD4+ T-cell clone from an NOD mouse immunized with this peptide (D. Healey and A. Cooke, personal communication).

**T-cell clones reactive to other β-cell antigens.** Other β-cell proteins have also been identified as T-cell antigens. T-cell reactivity to heat shock protein (HSP) was reported by Elias et al. (40), and HSP65 reactive T-cell lines were found to induce disease in NOD mice. Further, vaccination with an HSP65 peptide was found to protect against development of diabetes in NOD mice (41). Work from two laboratories indicated that T-cell reactivity to GAD65 or several GAD65 peptides could be detected in spleno-
ments in which CD4+ T-cell clones, specific for insulin in the presence of NOD APCs, were found to be capable of destroying islet allografts and rat or human xenografts (D.W., R. Gill, unpublished observations). Because these T-cell clones cannot recognize antigen on allo or xeno MHC, the results indicate that β-cell destruction by CD4+ clones can occur as a consequence of recognition of the antigen on a cell other than the target β-cell, a finding that is much in keeping with the observations of Lo et al. (45) in studies on antigen presentation in chimeric mice.

There has been increasing interest in the role of cytokines in T-cell pathogenesis, particularly in those produced by Th1 T-cells versus those produced by Th2 T-cells. The current paradigm is that cytokines such as IL-4 and IL-10, produced by Th2 T-cells, have the capacity to both counteract the actions of and downregulate the production of Th1 products, such as IFN-γ, leading to an amelioration of the destructive process. Although this is an attractive concept and there have been experiments in which administration of Th2 T-cell cytokines to NOD mice have resulted in protection from diabetes (46,47), it is also true that manipulations such as transgene-mediated production of IL-10 by β-cells have resulted in acceleration of the development of diabetes in NOD mice (48). The in vivo activities of islet-specific T-cell clones of either Th1 or Th2 phenotypes have just begun to be assessed, and thus far, the results provide little clarification. In the first report of Th2 T-cell lines from NOD mice, Healey et al. (33) observed that in comparison with a Th1 line that rapidly caused diabetes and extensive insulitis in neonatal NOD recipients, animals injected with a Th2 line did not become diabetic within the 30-day period of the experiment and had a nondestructive peri-ductal infiltrate. In a set of experiments by Katz et al. (49), in which mice transgenic for the T-cell receptor of the diabeticogenic CD4+ T-cell clone BDC-2.5 (13) were used as donors for transfer of in vitro activated spleen cells to recipient mice, it was observed that spleen cells activated under Th1-promoting conditions transferred diabetes with good efficiency. On the other hand, spleen cells activated under Th2-promoting conditions were not capable of inducing diabetes but were also unable to protect mice from the actions of Th1 cells. In a third investigation, it was found that both Th1-like and Th2-like insulin-specific T-cell clones could transfer diabetes and that a clone that could be switched to either a Th1 or a Th2 phenotype in vitro was diabeticogenic under both conditions (D.W., unpublished observations). Thus, it is not yet clear that Th2 T-cells can protect against diabetes in the NOD mouse.

Finally, there is the issue of autoantigen specificity of T-cell clones. Diabeticogenicity has been demonstrated for T-cell lines and clones reactive to undefined islet antigens and, in a few instances, to defined β-cell antigens. It is apparent that T-cell reactivities to a variety of islet cell proteins arise during the development of disease, and some of them have been detected in or isolated from mice as young as 4 weeks. There is extensive evidence from the studies of Bergman and Haskins (35, unpublished results) and a strong indication from the work of Shimizu et al. (27) as well as from the findings with human T-cell clones (10), that an important antigen or group of antigens is associated with the β-granule membrane. In these studies, no antigenicity was found in soluble supernatant or subcellular cytosolic fractions. On the other hand, T-cell specificities have now been demonstrated for several known β-cell proteins, and in some instances, treatment with these antigens under tolerizing conditions can ameliorate or inhibit disease. The important question that remains to be answered is whether there is any one T-cell autoantigen that is dominant in the initiation of disease and whether identification of that antigen will have therapeutic implications. The induction of antigen-specific tolerance in autoimmune disorders is being investigated by many labs, and evidence to date suggests that at least in the case of IDDM, there may be numerous antigens, including insulin and GAD, administered by various routes, that can prevent or inhibit disease in highly controlled, genetically identical mice. There are also many studies indicating that a wide variety of relatively nonspecific methods—including treatment with antibodies to several T-cell molecules and inhibitors or upregulators of cytokines—are efficacious in animals (6). Thus, it is difficult to predict at this time whether an antigen-specific approach will be better in the highly diverse human population than other approaches that are broader in spectrum. To return to the first part of the question regarding the importance of identifying an initiating antigen, it is improbable that every possible autoantigen in the islet β-cell would have the same potential for invoking a primary immune response; therefore, it seems likely that one antigen or group of antigens is involved in the initiation of disease. Identification of this antigen could contribute much in terms of gaining an understanding of how the disease process begins. Part of what has been gained from investigating islet antigens such as insulin and GAD lies in the fact that autoantibodies to these proteins have some value as predictive markers of disease. Since the ultimate approach to treating IDDM would be the rescue of islet function before autoimmune destruction begins or becomes advanced, improving the early diagnosis of IDDM is a high priority goal for clinicians and researchers, and it is in this regard that the identification of a dominant disease-initiating antigen is the most important.

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