Prospective study of lymphocyte subsets in subjects genetically susceptible to Type 1 (insulin-dependent) diabetes

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Summary. A prospective study of lymphocyte subsets has been carried out for 18 months in 58 healthy first-degree relatives of Type 1 (insulin-dependent) probands. Subjects selected for presence or absence of islet cell antibodies included 10 with complement-fixing islet cell antibodies, 10 with conventional islet cell antibodies and 38 without islet cell antibodies. Immunoregulatory and effector lymphocyte subsets, and in particular activated T-cells, were investigated using a panel of monoclonal antibodies. The results showed no significant changes in total T, helper, suppressor/cytotoxic cells or K/NK cells. Activated T-cells were observed at least once in 22 subjects using the 4F2 monoclonal antibody and in 11 using the Tac antibody. Seven subjects had 4F2-positive cells on repeated occasions and one twice showed Tac-positive cells. Fluctuations and/or loss of islet cell antibodies were observed

during follow-up. There was no correlation between presence of activated T-cells and either islet cell antibody status or HLA haplotype sharing with the diabetic proband. On the other hand, a significant correlation was observed between HLA-DR3 positivity of subjects and the occurrence of activated T-cells (both 4F2-positive and Tac-positive). We conclude that subjects with HLA-DR3 may be especially prone to T-cell activation. As none of the 'high risk' individuals developed diabetes in the course of follow-up, the relevance of these observations in the pathogenesis of Type 1 diabetes needs more prolonged investigation.

Key words: Type 1 diabetes, pre-diabetic state, genetic susceptibility, lymphocyte subpopulations, activated T-cells.

It is now well recognized that abnormalities in lymphocyte subpopulations are present in patients with newly diagnosed Type 1 diabetes. Alterations in the peripheral blood include the presence of activated DR-positive T-cells [1, 2], the decrease of suppressor cell number and function [3, 4] and an increased number and function of killer cells [5, 6].

It is still unclear what is the meaning of such abnormalities and whether they are primarily involved in the pathogenesis of the disease or are merely a secondary phenomenon present at the time of onset. One possible approach to this question is a prospective study in genetically and immunologically susceptible, but otherwise normal, first-degree relatives of Type 1 diabetic probands.

The Barts-Windsor-Middlesex Family Study, which was started in 1978, has clearly indicated, by regular testing of unaffected relatives of diabetic children, that humoral autoimmune phenomena may exist for years in some of those who have inherited the 'diabetogenic genes' [7, 8]. In order to investigate the role of cell-mediated immune phenomena in the prediabetic period, a

prospective study of lymphocyte subsets was started in 1982 by selecting a group of first-degree relatives in our family study.

The aim of this study was to define whether abnormalities of the immunoregulatory/effector cell subsets can occur in individuals susceptible to Type 1 diabetes, and whether such changes are persistent and can be regarded as markers for an ongoing autoimmune process directed against the β cells.

Subjects and methods

Subjects

Fifty-eight first-degree relatives of Type 1 diabetic probands entered the study. They were selected as follows: group A: 10 subjects (eight siblings and two parents) whose sera showed complement-fixing islet cell (CF-ICA) on at least one occasion; group B: 10 subjects (six siblings and four parents) who had conventional ICA (ICA-IgG) on one or more occasions; group C: 38 subjects (17 siblings and 21 parents) who, repeatedly tested for 3 years, were always negative for ICA-IgG and CF-ICA.

Fig. 1. **A** The lymphocyte subsets (expressed as percentage of total mononuclear cells) in the 18-month-prospective study in the three groups of subjects. □: CF-ICA; ▨: ICA-IgG; ▤: ICA-negative (ICA-neg). Differences between groups are not significant. **B** The proportions of activated T-cells (4F2-positive and Tac-positive; expressed as percentage of total mononuclear cells) in the three groups of subjects. Only specimens with percentages >2SD above the normal mean (shaded area) are represented. 0 = the mean values of activated T-cells in subjects who repeatedly showed these cells in the peripheral blood

All subjects were HLA-A, -B, -C and -DR typed [9]; nine siblings did not share HLA haplotypes with the diabetic probands (two in group B and seven in group C).

The blood glucose levels were within the normal range in all subjects at the time of entry into the study.

Lymphocytes

Blood peripheral mononuclear cells were collected approximately every 4–5 months over a period of 18 months. Cells were obtained by centrifugation of venous blood on Lymphoprep (Nygaard, Oslo, Norway), washed twice in Hank's balanced salt solution before being phenotyped.

Procedures

Cells (3.0×10^5 /tube) were incubated for 30 min at 4°C separately with 50 µl of the appropriate dilution of each of the following monoclonal antibodies: UCHT 1 [10], for total T-cells; UCHT 4 [11] for suppressor/cytotoxic T-cells (analogous to OKT8 or Leu 2a); Leu 3a for helper cells (Becton Dickinson, Sunnyvale, California, USA); H25 [12] for K/NK cells; 4F2 [13] for activated T cells; anti-Tac [14] for activated T-cells (receptor for the T-cell growth factor).

After incubation, cells were washed twice in Hank's solution and incubated with fluorescein-conjugated rabbit anti-mouse antiserum for 30 min at 4°C. Cells were then washed again twice in Hank's solution and for each of the monoclonal antibodies tested the percentage

Table 1. Relationship between presence of activated T-cells and lymphocyte subsets

Specimens groups by presence of activated T-cells	Number of specimens	Lymphocyte subsets as proportion of mononuclear cells		
		Total T-cells (%)	Helper/ suppressor ratio (%)	K/NK (%)
Activated T-cells present by the following criteria:				
> 15% of cells 4F2- positive ^a	22	62 ± 16	1.4 ± 0.5 ^b	16 ± 10
> 10% of cells Tac- positive ^a	11	52 ± 16	1.5 ± 0.9 ^b	25 ± 13 ^c
Activated T-cells absent by both tests	129	63 ± 7	1.8 ± 0.6	9 ± 8

^a 4F2 > 15% and Tac > 10% represent 2 SD above the mean proportions of mononuclear cells positive in normal subjects using the corresponding antibodies; ^b $p < 0.01$; ^c $p < 0.05$ using Mann-Whitney U test

of cells positive for rim fluorescence was determined using a Leitz inverted fluorescence microscope (Leitz, Wetzlar, FRG) and a Hamamatsu plate (Medicell International, London, UK), which allows the use of very low cell concentration [15].

Islet cell antibodies (ICA-IgG and CF-ICA) were measured on sections of human pancreas (group 0) as described previously [16].

Results

Minimal variations of immunoregulatory/effector cell subpopulations (helper, suppressor/cytotoxic, K/NK) were observed over the follow-up period in the three groups of subjects studied (Fig. 1). Activated T-cells as evaluated by the anti-Tac and 4F2 antibody are also shown in Figure 1. Only 11 subjects (five parents and six siblings – one on two occasions) showed Tac-positive cells; 22 subjects (10 parents and 12 siblings – seven on two occasions) had 4F2-positive cells. When Tac-positive cells were present, 4F2-positive cells were not detectable. However, four subjects with Tac-positive cells had shown 4F2-positive cells on previous occasions.

Activated T-cells (either 4F2-positive or Tac-positive) appear equally distributed in the three groups of subjects (Fig. 1).

During the 18 months of follow-up, CF-ICA and ICA-IgG fluctuated in both group A and group B; however, CF-ICA and ICA-IgG did not appear in group C during follow-up. Also, taking into consideration the fluctuations of ICA, there was no correlation between presence of activated T-cells and ICA. Thus only two out of 11 subjects with Tac-positive cells and six out of 22 subjects with 4F2-positive cells were positive for CF-ICA and/or ICA-IgG in the same blood sample.

Table 2. Relationship between activated T-cells and the HLA-DR phenotype

HLA	4F2 ^a		Tac ^a	
	> 15%	< 15%	> 10%	< 10%
DR3	14	11	9	16
DRX ^b	8	25	2	31
	$p < 0.05^c$		$p < 0.02^c$	

^a 4F2 > 15% and Tac > 10% represent values 2 SD above the normal mean. Figures represent numbers of subjects in each group who were positive for activated T-cells on at least one occasion (on left), or negative on every occasion (on right); ^b DRX = any DR allotype except DR3; ^c χ^2 test (with Yates' correction)

The relationship between the presence of activated T-cells and other lymphocyte subsets is shown in Table 1. When activated T-cells are present, abnormalities of lymphocyte subpopulations are observed, including a decreased helper/suppressor-cytotoxic ratio and increased percentage of K/NK cells.

Finally, no correlation was observed between the sharing of HLA haplotype with the diabetic proband and activated T cells, whereas a significant correlation was found between HLA-DR3-positive subjects and the occurrence of activated T-cells (4F2 and Tac) (Table 2).

None of the subjects studied developed diabetes during the follow-up period.

Discussion

This 18-month prospective study of lymphocyte subsets in first-degree relatives of Type 1 diabetic probands has demonstrated minimal variations in subpopulations of the immunoregulatory/effector cells, whereas activated T cells were observed in some individuals. The main finding is that HLA-DR3 positive individuals may be more prone to T-cell activation. This observation may be relevant in the wide spectrum of autoimmune diseases characterized by an association with HLA-DR3 and activated T cells (i.e. Graves' disease, Type 1 diabetes, myasthenia gravis) [17]. It has been suggested that either a gene associated with HLA-DR3 may predispose to autoimmunity [18] or, as the autoantigens involved in the various HLA-DR3 associated diseases are different, the preponderance of HLA-DR3 may reflect a relative ease of induction of aberrant DR in epithelial cells of individuals expressing these antigens [19]. The possibility that HLA-DR3 strongly influences the cell-mediated immune response is also suggested by data showing increased number of plaque-forming cells in culture from HLA-DR3 subjects [20]. Therefore our observation of a significant correlation between DR3 and activated T cells support this concept.

However, since none of the "high risk" individuals has developed diabetes in the past 3 years, the relevance of these observations in the pathogenesis of Type 1 diabetes needs more prolonged investigation of these sub-

jects. The fact that ICA may become undetectable in genetically predisposed subjects [21] fits well with the present observation that presence of activated T cells can also fluctuate, suggesting that susceptible individuals do possess natural protective mechanisms against autoimmune damage such as enhanced suppressor T-cell function or anti-idiotypic response to ICA or to autoreactive T cells [22]. In this context, it is of interest that when activated T cells were found, the helper/suppressor ratio was strongly reduced due in particular to a reduction of cells carrying the suppressor/cytotoxic phenotype.

In conclusion, future efforts must concentrate on the identification of both the crucial destructive mechanisms and the potentially important regenerative processes which may counteract β -cell destruction.

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