Importance of persistent cellular and humoral immune changes before diabetes develops: prospective study of identical twins

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Abstract

Objectives—To determine the pattern of cellular and humoral immune changes associated with insulin dependent diabetes before diabetes develops.

Design—Prospective study over 10 years of 25 non-diabetic identical twins of patients with insulin dependent diabetes. The non-diabetic twins were followed up either till they developed diabetes or to the end of the study.

Setting—Teaching hospital.

Subjects—25 non-diabetic identical cotwins of patients with diabetes; 46 controls of the same sex and similar age tested over the same period. Of the 25 twins (total follow up 144 patient years), 10 developed diabetes (prediabetic twins); the remainder were followed up for a mean of 7.7 years.

Main outcome measures—Results of glucose tolerance tests or fasting blood glucose concentrations at each sample point. Measurements of activated T lymphocytes, expressing the HLA-DR antigen, islet cell antibodies, and insulin autoantibodies in samples.

Results—All 10 prediabetic twins had both cellular and humoral changes initially and in most samples before diabetes was diagnosed (activated T lymphocytes in 39/40, islet cell antibodies in 45/47, and insulin autoantibodies to islet cells and insulin were detected infrequently (in 8/54, 6/69, and 0/69 samples, respectively). The combination of cellular and humoral (islet cell antibodies or insulin autoantibodies) immune changes were detected in all 10 of the prediabetic twins but in only one of the 15 non-diabetic twins ($P < 0.001$). The positive predictive value in this cohort of increased percentages of activated T cells and the presence of antibodies to islet cells or insulin on two consecutive occasions was 100%.

Conclusion—Most of the twins had cellular or humoral immune changes at some stage. A combination of cellular and humoral immune changes and their tendency to persist is highly predictive of insulin dependent diabetes and distinguishes twins who develop diabetes from those who do not.

Introduction

Insulin dependent diabetes mellitus is due to destruction of the insulin secreting islet cells of the pancreas, probably by T lymphocytes that recognise an antigen specific for β cells. The disease is believed to be induced by an environmental event operating in a genetically susceptible person. At diagnosis many patients with insulin dependent diabetes show both cellular and humoral immune changes in their peripheral blood, including the production of autoantibodies to islet cells and insulin,^{1,2} and activation of T lymphocytes.³ These immune changes can be detected

many months before the onset of diabetes, suggesting that the disease process is prolonged.⁴ Genetic susceptibility to insulin dependent diabetes is conferred by HLA genes and probably also by non-HLA genes, as yet ill defined.⁵ Studies in identical twins of diabetic patients—in contrast to family studies—would elucidate the potential importance of markers for diabetes in people who are genetically susceptible to the disease.

In 1982 we began a prospective study of a cohort of non-diabetic identical twins of patients with insulin dependent diabetes. Samples from the twins were obtained to determine T lymphocyte activation, through expression of HLA-DR, and the presence of autoantibodies to islet cells and insulin, these immune changes being found in such twins.^{2,6} The twins were followed up either till they developed diabetes or to the end of the study. Our aim was to define the extent and pattern of immune changes before diabetes develops and whether the changes differ according to whether the cotwin develops diabetes. We were particularly interested in whether these cellular and humoral immune changes were intermittent or persistent during the prediabetic period.

Patients and methods

We followed up a consecutive series of non-diabetic identical cotwins of patients with insulin dependent diabetes. The cotwins were referred within four years of the diagnosis in the index twin and seen between June 1982 and January 1992. The period over which the twins who entered the study were ascertained was from June 1982 to June 1990. Monozygosity was established in all the twin pairs as previously described.⁷ All the twins had oral glucose tolerance tests (glucose was given as 75 g or 1.75 g/kg, whichever was the less) to confirm that they were not diabetic both initially and at intervals thereafter.⁸ Each twin on every occasion had a fasting blood glucose concentration of less than 6.7 mmol/l to exclude overt diabetes. The intention was to test twins at least annually and, if possible, up to three times a year if they showed immune or metabolic changes. Twins were followed up to January 1992, when either they had developed diabetes, as previously defined, or they were non-diabetic.^{9,9} Control subjects ascertained from the local community and not attached to the hospital were also tested on a single occasion during the same period as the twins. These control subjects were selected to achieve a similar distribution for age and sex to the twins; they had no family history of diabetes, were taking no drugs, and had no clinical sign of illness. The subjects or their parents gave informed consent, and the study was approved by the ethics committees at Westminster Hospital and King's College Hospital.

Twenty five non-diabetic twins fulfilled the criteria

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of entry to the study and were followed up prospectively from the diagnosis of the index twin for a total of 144.4 patient years until either 1 January 1992 or until they developed insulin dependent diabetes. Seventeen twins were ascertained 1.0 or less years from the diagnosis of diabetes in their twin, three 1.1 to 2.0 years, three 2.1 to 3.0 years, and two 3.8 years. During follow up 10 twins developed diabetes (referred to as prediabetic twins). At the end of the study we therefore had two groups: 10 prediabetic twins (mean (SD) age on entry 11.9 (4.7) years; five male) and 15 non-diabetic twins (mean age on entry 17.5 (8.1) years; eight male); there was no difference in the age at entry between the two groups. There was also no significant difference in the mean ages at diagnosis of the index twins in the two groups (11.7 (4.5) years in prediabetic group *v* 16.1 (8.7) years in non-diabetic group). Time of ascertainment from the diagnosis of diabetes in the index twin was shorter in the prediabetic twins than in the non-diabetic twins (median 0.2 (range 0-1.8) *v* 1.0 (0.2-3.8), respectively; *P*=0.05).

At entry into the study all 10 prediabetic twins lived with their index twin, as did eight of the 15 non-diabetic twins. At the end of the study one of the 10 prediabetic twins and five of the 15 non-diabetic twins were living apart. The prediabetic twins were followed up for a median of 29 months (range 7-79) and were tested on a total of 56 occasions before they developed diabetes. When their diabetes was diagnosed nine twins had symptoms and a raised blood glucose concentration (range 11.8-26.4 mmol/l). The remaining twin had an increased fasting blood glucose concentration (7.1 mmol/l) but no diabetic symptoms, though she required insulin treatment six months later for hyperglycaemia and ketonuria. The 15 non-diabetic twins were followed up for a median of 102 months (range 31-143) and were tested on a total of 75 occasions.

The twins were compared with 21 non-diabetic healthy controls (mean age 23 years, range 7-42 years; 10 male) for percentages of activated T lymphocytes and with 25 non-diabetic healthy controls (mean age 18 years, range 3-24 years; 12 male) for autoantibodies to islet cells and insulin. Cells and serum samples from the controls were obtained over the same time as those of the twins.

Cells and serum samples were obtained when possible from each subject on every occasion. Sometimes, however, the material obtained was not sufficient for analysis. Peripheral blood mononuclear cells were purified from venous blood and stored under liquid nitrogen until analysis. Analysis was performed on batched samples by a single observer (LA), who was blinded to the clinical status of the subjects; samples were analysed on four occasions in 1986, 1990, 1992, and 1993. Serum samples were stored at -20°C, and analysis for antibodies was performed on batched samples in 1992 and 1993 by observers blinded to the clinical status of the subjects.

ACTIVATED T LYMPHOCYTES

T lymphocytes, purified from peripheral blood mononuclear cells by a rosetting technique, were analysed for surface expression of HLA-DR (present on activated T cells); this was detected with a fluorescein labelled HLA-DR antibody as previously described.³ This antibody obtained from clone L243 (Beckton Dickinson, Oxford) recognises a non-polymorphic HLA-DR (class II) determinant.¹⁰ At least 400 cells were counted and the results expressed as the percentage of fluorescein stained T cells minus 2% to allow for contamination by B lymphocytes and monocytes, which usually bear HLA-DR determinants.³ Values greater than 2 SD above the mean obtained in controls were regarded as positive.

ISLET CELL ANTIBODIES

Undiluted serum samples from twins were screened for islet cell antibodies. The presence of antibodies was established by testing serum by indirect immunofluorescence on a fresh cryofixed human pancreas (blood group O).¹¹ The titre of each sample was determined by serial doubling dilution. The same pancreas, reagents, and incubation conditions gave end point titres of 32 with the putative standard islet cell antibody (equivalent to 80 Juvenile Diabetes Foundation (JDF) units) that was assessed for an immunology and diabetes workshop. The results are presented both as JDF units and as positive or negative; values greater than 4 JDF units were regarded as positive.

INSULIN AUTOANTIBODIES

Insulin autoantibodies were measured by a liquid phase radioligand binding assay, with displacement using unlabelled insulin as previously described.¹¹ Corrected binding was determined by subtracting the binding after incubation with excess unlabelled insulin from the binding with label alone. Corrected binding values greater than 3 SDs above the mean obtained in normal serum were regarded as positive for insulin autoantibody. The interassay coefficient of variation was 8.6%.

STATISTICAL ANALYSIS

Results are expressed as means (SD) or medians (ranges) when appropriate. Initial and final values in all tests were compared between and within groups. In addition, percentages of activated T cells and islet cell antibodies titres (JDF units) during follow up were compared using the means of determinations for each twin, which avoids potential bias due to different numbers of samples and different lengths of follow up. Changes occurring during the study were analysed by comparing the first and last samples from each twin with the Wilcoxon rank sum test for matched pairs.

Possible relations in changes with time from diagnosis in the index twin or time to diagnosis in the prediabetic twin were analysed with Spearman's rank correlation coefficient on pooled determinations from all of the twins and, when sufficient determinations were available, serial determinations from an individual twin in order to obtain a rough estimate of any relation in immune changes with time. Apart from the comparison of the first and last sample, all analyses were performed using Student's *t* test on untransformed or log transformed data as appropriate.

Standard Kaplan-Meier actuarial life table methods were used to calculate the cumulative risk of twins developing insulin dependent diabetes from the initial sample.⁹ The log rank test was used to compare the distribution of risk of diabetes according to initial immune status. We estimated (a) the positive predictive value by calculating the number of twins with abnormal test results who later developed diabetes as a percentage of the overall number of twins who had abnormal results; (b) the sensitivity by dividing the number of twins with high results who developed diabetes by the overall number of twins who developed diabetes; (c) the specificity by dividing the number of twins with normal results who did not develop diabetes by the total number of twins who did not develop diabetes. As immune changes are unlikely to be static, the calculated values were determined on the first sample taken, and values estimated for consecutive results were estimated on the first and second sample tested.

Changes in the study were considered to be significant at *P*<0.05.

Results

ACTIVATED T LYMPHOCYTES

Control subjects—Twenty one controls had mean proportions of activated T cells of 2.1% (1.0%). Percentages greater than the mean and 2 SD of control values—that is, greater than 4.1%—were considered to be raised in the 25 non-diabetic twins (5.1% (4.1%)), 10 of whom later developed diabetes.

Prediabetic twins—Activated T cells in the 10 prediabetic twins were initially 8.6% (2.5%) and at the final sample 8.2% (2.7%). The mean of the average value for each twin throughout the prediabetic period was 8.3% (2.2%). Percentages of activated T cells in prediabetic twins were significantly higher than those in the non-diabetic twins initially ($P \leq 0.002$), throughout the study ($P < 0.00001$), and at the final sample ($P < 0.00003$). All values in prediabetic twins—that is, at the initial sample, throughout the study, and at the final sample—were significantly higher than those observed in control subjects ($P \leq 0.0005$). Percentages of activated T cells greater than control values ($> 4.1\%$) were found in all 10 prediabetic twins initially and in a total of 39 out of 40 samples before diabetes developed. Increased percentages of activated T cells persisted in nine of the 10 prediabetic twins up to diagnosis; in the remaining twin the percentage fell to just within the normal range on one occasion (fig 1).

Non-diabetic twins—Levels of activated T cells in these 15 twins were initially 3.9% (3.9%), and at the final sample 2.1% (1.7%); the mean of the average value for each twin throughout the study period was 2.1% (1.6%). The values in these non-diabetic twins were significantly higher than in control subjects initially ($P < 0.0005$) but not subsequently. Increased percentages of activated T cells were found initially in six of the 15 twins who remained non-diabetic. Only eight of the total number of 54 samples taken overall showed increased percentages of activated T cells. None of the twins showed persistent T cell activation over the period of study. Even twins who had activated T cells at some stage, but remained non-diabetic, had lower percentages of activated T cells than the prediabetic twins (7.2% (3.2%) *v* 8.9% (2.8%).

Changes with time—In prediabetic twins regression analysis of changes in percentages of activated T lymphocytes throughout the study, either in individual subjects or in the group, did not show significant trends; there was also no significant difference between the first and last samples. In the non-diabetic twins

FIG 1—Percentages of activated T lymphocytes according to period of follow up from diagnosis of diabetes in index twin until onset of diabetes in prediabetic twins ($\blacktriangle$) or end of study in twins remaining non-diabetic ($\circ$). Upper limit of normal values (mean (2SD)) is shown by solid line

FIG 2—Titres of islet cell antibodies in JDF units according to period of follow up from diagnosis of diabetes in index twin until onset of diabetes in prediabetic twins ($\blacktriangle$) or end of study in twins remaining non-diabetic ($\circ$). Sensitivity of assay (< 5 JDF units) is given by solid line

there was also no significant trend throughout the study either in individual subjects or in a group. There was, however, a significant decrease in the values of activated T cells in these twins when the first and last tests were compared ($P < 0.05$).

Predictive values—The risk of diabetes by life table analysis after 10 years from the initial sample in the non-diabetic twins was 63% (95% confidence intervals 39% to 86%) in those with increased percentages of activated T lymphocytes compared with 0 in those without such increases ($P < 0.01$). The positive predictive value of increased percentages of activated T cells in this cohort was 63%, with a sensitivity of 100% and a specificity of 60%. The positive predictive value of increased levels of activated T cells on two consecutive occasions was 100%, with a sensitivity of 89% and a specificity of 100%.

ISLET CELL ANTIBODIES

Control subjects—None of the controls had islet cell antibodies.

Prediabetic twins—Antibodies were found initially in nine of the 10 prediabetic twins; eight had them in all subsequent samples before the onset of diabetes and one had them on one of two occasions before diagnosis. Islet cell antibodies were detected in 45 of the total of 47 samples taken before the onset of diabetes in this group (fig 2). There was no pattern to the titre of antibodies during the prediabetic period—that is, there was no significant difference in the median titre between the initial and final sample (45 (range < 5 –80) and 49 (< 5 –80) JDF units, respectively). The median titre of islet cell antibody in positive samples throughout the study was 64 (< 5 –320) JDF units. There was no correlation between titres and the proportions of activated T cells.

Non-diabetic cotwins—Antibodies were found in one of the 15 non-diabetic twins initially and in two twins during follow up (fig 2). One twin had antibodies on four out of six occasions and the other two twins, on one out of three and one out of eight occasions. Islet cell antibodies were detected in six out of 69 samples taken throughout the study. None of the twins showed persistent antibodies during the study period. There was no significant difference in the median titre of the initial (< 5 (< 5 –34) JDF units) and final samples (< 5 (< 5 –9) JDF units). The median titre of islet cell antibodies in positive samples was 7 (6–34) JDF units. There was a significant tendency for prediabetic twins who had islet cell antibodies to have a higher titre than

Case No	Sex	Age of index twin at onset of diabetes (years)	Follow up from onset in index twin (years)*	Total No of tests during study	Mean activated T cells during study (%)	Mean islet cell antibody titre during study (JDF units)	Proportion of tests positive for insulin autoantibodies
Prediabetic twins:							
1	M	16.6	2.3	2	9.4	41	0/2
2	M	12.6	1.8	5	9.1	13	0/4
3	F	9.9	2.6	3	4.1	55	0/1
4	M	10.0	1.5	4	10.5	64	1/2
5	F	8.6	3.6	3	8.6	57	2/2
6	F	9.0	0.6	2	8.3	13	0/2
7	F	18.5	5.0	10	6.9	59	1/7
8	M	11.0	3.8	12	10.8	117	0/9
9	F	2.1	0.6	1	5.5	<5	1/1
10	F	13.4	6.6	12	9.5	63	1/12
Non-diabetic twins:							
11	M	12.6	7.2	6	1.5	<5	0/5
12	F	23.5	7.4	5	0.7	<5	0/4
13	M	19.0	8.5	6	0.8	<5	0/5
14	M	22.4	8.5	7	1.6	10	0/6
15	F	23.6	9.2	7	3.4	<5	0/6
16	F	26.9	11.0	3	3.6	6	0/3
17	M	4.4	11.9	4	5.4	<5	0/4
18	M	15.4	10.2	9	3.0	<5	0/8
19	M	10.9	10.8	5	3.5	<5	0/5
20	F	10.9	11.6	6	4.7	<5	0/6
21	M	35.4	2.6	4	1.0	<5	0/4
22	F	6.8	2.8	3	1.8	<5	0/3
23	M	11.7	4.0	4	0.5	<5	0/4
24	F	12.0	4.2	3	0.5	<5	0/3
25	F	6.2	5.1	3	1.6	<5	0/3

*To estimate means, ICA titre of <5 JDF units was arbitrarily taken as 2.5 JDF units.

FIG 3—Life table analysis showing percentages (with SE) of identical twins who remained non-diabetic in years from initial blood sample according to whether they did (—) or did not (---) initially have increased percentages of T lymphocytes and either islet cell antibodies or insulin autoantibodies

those who did not develop diabetes ($P < 0.01$). A significant difference was also found in the mean values between the prediabetic twins (56 (<5–117) JDF units) and twins who did not develop diabetes (<5 (<5–34) JDF units; $P < 0.00005$).

Changes with time—Regression analysis of islet cell antibody titres in individual twins or in prediabetic and non-diabetic groups did not show significant trends.

Predictive values—The risk of diabetes by life table analysis for twins who were positive for islet cell antibodies in the initial sample was 90% (71% to 100%) compared with 7% (0 to 19%) in those without antibodies ($P < 0.0001$). The positive predictive value of islet cell antibodies in this cohort was 90%, with a sensitivity of 90% and a specificity of 93%. The positive predictive value of islet cell antibodies on two consecutive occasions was 100%, with a sensitivity of 70% and a specificity of 100%.

INSULIN AUTOANTIBODIES

Prediabetic twins—Insulin autoantibodies were found initially in two of the 10 twins and in five twins overall during the prediabetic period. They were found in six out of 42 samples taken before the onset of diabetes (table). One twin had autoantibodies in both samples obtained before the onset of diabetes, binding decreasing from 6.98% to 1.19%. Four twins had insulin autoantibodies on only one occasion—that is, in four out of 18 samples.

Non-diabetic cotwins—Insulin autoantibodies were not found in any of the 15 non-diabetic twins when they were tested initially or in any of the 69 samples taken during the study.

Changes with time—Regression analysis of insulin autoantibody values in individual twins or in prediabetic and non-diabetic groups did not show significant trends.

Predictive values—The risk of diabetes by life table analysis for twins who were positive in the initial sample was 100% compared with 37% (15% to 60%) in those without ($P < 0.0005$). The positive predictive value of insulin autoantibodies in this cohort was 100%, with a sensitivity of 20% and a specificity of 100%. The positive predictive value of the antibodies on two consecutive occasions was 100%, with a sensitivity of 10% and a specificity of 100%.

ACTIVATED T LYMPHOCYTES AND EITHER ISLET CELLS OR INSULIN AUTOANTIBODIES

Prediabetic twins—Increased percentages of activated T cells and either islet cell antibodies or insulin autoantibodies were found in all 10 twins initially and in 29 of the total 31 samples tested for both cellular and humoral changes. Results from two consecutive samples were available from seven twins, of whom six showed increased percentages of activated T cells and either islet cell antibodies or insulin autoantibodies.

Non-diabetic twins—Increased percentages of activated T cells and either islet cell antibodies or insulin autoantibodies were found in only one of the 15 non-diabetic twins tested initially, and this was the only positive sample out of 48 tested. Thus, there is a significant tendency for prediabetic twins to have both cellular and humoral immune changes as compared with non-diabetic twins (10/10 *v* 1/15 ($P < 0.001$)). None of the non-diabetic twins had increased percentages of activated T cells with either islet cell antibodies or insulin autoantibodies in two consecutive samples. Of the 15 non-diabetic twins, six had either increased percentages of activated T cells or islet cell antibodies at some stage during the study.

Predictive value—The risk of insulin dependent diabetes by life table analysis for twins who had increased percentages of activated T cells and islet cell antibodies or insulin autoantibodies was 92% (74% to 100%) compared with 0 in those without this combination ($P < 0.00001$) (fig 3). The positive predictive value of increased percentages of activated T cells and either islet cell or insulin antibodies in this cohort was 91%, with a sensitivity of 100% and a specificity of 93%. The risk of diabetes by life table analysis for twins who had increased percentages of activated T cells and islet cell or insulin antibodies in consecutive samples was 100% compared with 8% (0 to 24%) in those without this combination ($P < 0.0001$). The positive predictive value of increased percentages of activated T cells and islet cells or insulin antibodies on two consecutive occasions was 100%, with a sensitivity of 86% and a specificity of 100%.

Discussion

Although the destructive process in insulin dependent diabetes is thought to be cell mediated, prospective studies of predictive markers have concentrated on antibodies to islet cells and insulin rather than on cellular immune changes. Our findings are therefore interesting in showing that, at least in these twins, both cellular and humoral immune changes occur before diabetes develops and that they persist up to the clinical onset of diabetes. Twins who did not develop diabetes often had either cellular or humoral immune changes, but in contrast to prediabetic twins, only one of them had both changes concurrently, and then only once. Thus, the combination of cellular and humoral immune changes and their persistence distinguished twins who developed diabetes from those who did not.

An increased percentage of activated T lymphocytes may be found in several autoimmune diseases including diabetes^{12–16}; when present in twins of diabetic patients it is a highly sensitive marker for the development of diabetes. T cell activation was also observed in the twins who remained non-diabetic. In these non-diabetic twins the percentages of activated T lymphocytes were initially raised but fell to normal and were always significantly lower than in the prediabetic twins, as was the titre of islet cell antibodies. To our knowledge, no previous study has tested whether persistence of cellular or humoral immune changes predicts diabetes, probably because of the difficulty of purifying and storing peripheral blood mononuclear cells before analysis. A reassessment of

published studies, however, confirms the observation that immune changes, including islet cell autoantibodies^{14 17-19} and increased percentages of activated T cells,¹² can be present before diabetes develops. In one family study the positive predictive value of islet cell antibodies was greater when they were detected on three separate occasions than only once.²⁰ Our study of twins shows the persistence of both cellular and humoral immune changes before diabetes develops, as well as the predictive value of these changes when present simultaneously.

SCREENING FOR DIABETES

Although high titres of islet cell antibodies are strongly predictive of insulin dependent diabetes in twin and family studies, they are less predictive in the general population.^{11 20 21} Further characterisation of immune changes before diabetes develops is important, therefore, if the prediction of sporadic cases of diabetes is to improve. Studies of populations at high risk of disease, such as twins, enable identification of potential strategies for use in the general population. One approach is suggested by our results. Subjects can be identified from the general population by screening with a sensitive, but not specific, disease marker such as low titres of islet cell antibodies.²¹ They could then be tested repeatedly for both cellular and humoral immune changes to enhance the power of predicting diabetes. Our observations in twins suggest that three or more samples should be taken over at least two years to enhance prediction, though we have not systematically assessed the period of study required. The specificity of immune changes as predictors might be further improved by assessing responses to islet cell antigens, in particular glutamic acid decarboxylase and an unidentified polypeptide of 37 kDa.^{11 22-24} Antibodies to these antigens, particularly the 37 kDa antigen, may have predictive value in twins.¹¹ Preliminary evidence from prospective family studies suggests that antibodies to glutamic acid decarboxylase behave similarly to those to islet cells in that once detected they usually persist throughout the prediabetic period.^{24 25} Further studies of these cellular and humoral immune responses to islet antigens, as predictors of diabetes in twins, are in progress. Future studies will focus on the potential heterogeneity of antigen specific cellular and humoral immune responses in different populations and their comparative predictive value.

Twin pairs in which the index twin is under the age of 15 years at diagnosis have a greater risk of becoming concordant than those pairs in which the index twin is older.⁹ In our study the pairs who became concordant for diabetes tended to be younger at entry than those who remained discordant. Differences in concordance rates with age of diagnosis of the index twin are unlikely to be due to major environmental differences as most of the twins were living together at ascertainment. Any such difference might be, at least in part, genetically determined as diagnosis of diabetes is earlier in twin pairs with both HLA-DR 3 and 4 than in those with HLA-DR 3 or 4; twins with both HLA-DR 3 and 4 also have an increased concordance rate for diabetes.²³ In this study, as in others, relatives of diabetic patients who subsequently developed diabetes showed immune changes when first tested.^{24 26} They had presumably already been exposed to the environmental event that probably induces the immune changes. Since most of the twins, and other relatives who have been studied, are young at the time of ascertainment, the environmental event must have occurred in early childhood. We have previously proposed that the environmental event operates early in life and over a short time⁹; our current observations are consistent with this hypothesis. We did not detect intermittent immune changes or a progressive increase

Clinical implications

- Many patients with insulin dependent diabetes show specific cellular and humoral immune changes—namely, activated T lymphocytes and antibodies to islet cells and insulin
- This study examined these markers in 25 identical twins of diabetic patients according to whether they became diabetic themselves
- Activated T lymphocytes and either islet cell or insulin antibodies were detected persistently in all 10 of the twins who became diabetic but on only one occasion in one of the 15 twins who did not
- A combination of activated T lymphocytes and either islet cell or insulin antibodies predicts diabetes
- Testing for islet cell antibodies would be a sensitive but not specific marker to identify people at risk in the general population

in values in the time before diabetes developed; either of these findings would have been consistent with exposure to multiple environmental events.

CONCLUSIONS

Extensive evidence from twin, family, and population studies shows that immune or metabolic changes may herald the onset of diabetes in some subjects but not others.²⁷ The results of our current study suggest that an immune process may pursue a chronic progressive course of β cell destruction leading to diabetes or, alternatively, a similar process, presumably less intense and less extensive, may remit spontaneously without leading to diabetes.

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Survival differences in European patients with AIDS, 1979-89

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Abstract

Objectives—To examine the pattern of survival and factors associated with the outcome of disease in patients with AIDS.

Design—Inception cohort. Data collected retrospectively from patients' charts.

Setting—52 clinical centres in 17 European countries.

Subjects—6578 adults diagnosed with AIDS from 1 January 1979 to 31 December 1989.

Main outcome measures—Survival after the time of diagnosis.

Results—The median survival after diagnosis was 17 months, with an estimated survival at three years of 16% (95% confidence interval 15% to 17%). Patients diagnosed in southern Europe had a shorter survival, particularly immediately after the time of diagnosis, compared with patients diagnosed in central and northern Europe (survival at one year (95% confidence interval) 54% (52% to 56%) 66% (64% to 68%), 65% (63% to 66%), respectively. The three year survival, however, was similar for all regions. The regional differences in survival were less pronounced for patients diagnosed in 1989 compared with earlier years. Improved survival in recent years was observed for patients with a variety of manifestations used to define AIDS but was significant only for patients diagnosed with *Pneumocystis carinii* pneumonia. The three year survival, however, remains unchanged over time.

Conclusions—Survival of AIDS patients seems to vary within Europe, being shorter in southern than central and northern Europe. The magnitude of these differences, however, has declined gradually over time. Short term survival has improved in recent years, but the long term prognosis has remained equally poor, reflecting the fact that the underlying infection with HIV and many of the complicating diseases remains essentially uncontrolled.

Introduction

The survival of patients with AIDS may depend on a variety of factors including host factors, the pattern of diseases present, diagnostic routines, and therapeutic interventions. Improved survival of patients over time

has been reported, especially in patients with *Pneumocystis carinii* pneumonia.¹⁻⁸ This may be due to earlier diagnosis, improved treatment, and the introduction of secondary prophylaxis.⁹⁻¹¹ Antiretroviral treatment with zidovudine, which was introduced in 1987, may also have contributed to the observed improvement in survival.^{3,6,12}

We analysed the pattern of survival among 6578 patients from 17 European countries and assessed the association of clinical, demographic, and therapeutic factors on survival. Given the uniquely wide geographical scope of this study, we also focused particularly on possible regional differences in survival and explanations for them.

Subjects and methods

Fifty two centres in 17 countries participated in the study. Centres provided data on all patients (except for 23 Italian centres) diagnosed with AIDS between 1979 and 31 December 1989. The 23 Italian centres each enrolled only a predefined proportion of their patients according to month of birth; this selection was supervised by the Istituto Superiore di Sanita in Rome.

Information was collected from patients' charts on a standardised data collection form and included demography, serology (result of test for HIV antibodies), CD4 cell count within three months of diagnosis; time of initiation and permanent discontinuation of zidovudine; and time of diagnosis of opportunistic infections and malignancies and how these diagnoses were established. AIDS was defined according to the revised criteria from the Centers for Disease Control from 1987.¹³ Time variables were collected as month and year.

The collection of data was performed by retrospective review of case notes between May 1991 and August 1992. Members of the coordinating centre visited all major centres to ensure correct transferral of data from the patients' charts to the data collection form. All forms received by the coordinating centre were checked by scientific staff for logistical errors. A total of 6655 patients were enrolled. Seventy seven patients were excluded because the time of diagnosis of AIDS or time of latest follow up were not known, because clinical information was missing, or because the subject was under 16 years at the time of diagnosis.