Rapid communication

Loss of Ia-positive epidermal Langerhans cells at the onset of Type 1 (insulin-dependent) diabetes mellitus

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Summary. Immunocompetent antigen-presenting Langerhans cells were investigated in skin biopsies of 20 short-term Type 1 (insulin-dependent) diabetic patients and compared with 17 matched normal control subjects. Langerhans cells in epidermal sheet preparations were visualized with a monoclonal anti-HLA DR antibody using indirect immunofluorescence. A significant decrease of Langerhans cells/mm^2 body surface area was found in 10 patients immediately at the onset of diabetes compared to 10 patients with 6 months duration of diabetes and to normal control subjects (401 ± 30 vs 559 ± 43 vs 611 ± 33, $p < 0.01$ and $p < 0.002$). There was no significant difference in the number of Langerhans cells between patients with 6 months duration of diabetes and control subjects. Examination of the most likely precursor of Langerhans cells, the blood monocytes, indicated an increase of monocyte counts in Type 1 diabetic patients after 6 months duration (344 ± 37 cells/μl vs 191 ± 31 in control subjects, $p < 0.05$) and an inverse correlation between the number of Langerhans cells in skin with the number of monocytes in peripheral blood (at onset: $r = -0.73$, $p < 0.01$, after 6 months of diabetes: $r = -0.61$, $p < 0.05$). In addition, a positive correlation between Langerhans cells and daily insulin dose was noted in patients after 6 months of diabetes ($r = 0.76$, $p < 0.01$). The data suggest a loss of Langerhans cells in skin at the onset of Type 1 diabetes and that functional alterations of these and perhaps also other antigen-presenting cells may be involved in the pathogenesis of Type 1 diabetes.

Key words: Epidermal Langerhans cells, Type 1 (insulin-dependent) diabetes, antigen presentation, autoimmunity, monocytes.

It is now commonly accepted that autoimmunity is involved in the pathogenesis of Type 1 (insulin-dependent) diabetes and T-cell activation and alterations of T-lymphocyte subsets may be present in the peripheral blood of newly-diagnosed diabetic patients [1]. It has not been established, however, whether alterations of T-cells are the primary event in the disease. T-cell abnormalities, e.g. might be secondary to defects in the antigen-presenting cell system.

As recently reviewed by Stingl and co-workers, epidermal Langerhans cells (LC) are an integral part of the immune system in man and represent the antigen-presenting cells of the skin [2]. These bone marrow-derived dendritic cells have on the surface Ia, i.e. immune associated, HLA-DR alloantigens, as well as Fc (fragment c of immunoglobulins) and complement receptors. Evidence has been accumulated that epidermal LC can produce the monokine interleukin 1 and are needed for the generation of the T-cell dependent immune response originating in the epidermis. They are the only cells in the normal epidermis

that express and synthesize the Ia-antigen; stains for the presence of these surface markers can therefore be used to detect and enumerate LC. It has been shown that quantitative and qualitative changes of epidermal LC are present in several autoimmune diseases and that LC numbers are low or absent in the epidermis following ultraviolet irradiation or local and generalised corticosteroid therapy [3–6]. The present study was designed to investigate epidermal LC at the onst of Type 1 diabetes mellitus and 6 months thereafter.

Subjects and methods

Subjects

Twenty patients hospitalised at the City Hospital Munich Schwabing with short-term Type 1 diabetes mellitus were included in the study. Ten patients (6 women, 4 men) were skin biopsied at the onset of diabetes within the first days after diagnosis (13 ± 4 days). In ten patients (3 women, 7 men) the biopsies were performed 6 months after onset. According to diabetes duration the patients were divided

into two groups (T_0 and T_6). Group T_0: mean age 25 ± 1 years, HbA_{1c} $10.9\pm0.5\%$ (normal range $<6\%$), body mass index (BMI) 20.40 ± 0.70 kg/m^2; insulin dose 0.53 ± 0.10 units·kg^{-1}·day^{-1}; postprandial glucose 10.55 ± 0.88 mmol/l. Group T_6: mean age 25 ± 2 years; HbA_{1c} $8.3\pm0.9\%$; BMI 21.07 ± 0.26 kg/m^2; insulin dose 0.40 ± 0.05 units·kg^{-1}·day^{-1}; postprandial glucose 10 ± 1.10 mmol/l. Control skin biopsies were obtained during the same time period from 17 healthy volunteers (9 women, 8 men, mean age 28 ± 1 years, BMI 21.42 ± 0.43 kg/m^2) who exhibited normal glucose tolerance and HbA_{1c} levels. All probands enrolled in the study had neither received immunosuppressive agents or corticosteroids during the preceding year nor had they a history of recent prolonged exposure to sunlight. Informed consent was obtained from all subjects participating in the study and approval was given by the local Ethical Committee.

Skin biopsies

Four mm biopsy specimens were obtained from the sunprotected inner aspect of the upper arm. Local anaesthesia with 1% Xylocaine was used.

Epidermal sheet preparation

Skin specimens were floated dermis-side down on 0.5 molar phosphate-buffered ammonium-thiocyanate for 40 min at 37° C. The epidermal sheets were then easily separated from the underlying dermis, fixed by immersion into acetone for 20 min at room temperature, and rinsed in phosphate buffered saline (PBS, pH 7.2) for 1 h [7].

Identification of Ia-positive cells in the epidermis

Ia-positive cells in epidermal sheet preparations were visualized by using a slight modification of an immunofluorescence technique described previously [8] (see also acknowledgements). In brief, epidermal sheets were incubated for 16–18 h at 4° C with a mouse monoclonal anti-human Ia-antibody (Anti-HLA-DR, clone L243, Becton Dickinson, Heidelberg, FRG) diluted 1:3 in PBS/BSA (bovine serum albumin 1%). After extensive washing in PBS for 1 h, the sheets were incubated for 90 min at 37° C with 0.1 ml of a 1:10 diluted fluorescein isothiocyanate-conjugated goat anti-mouse IgG (Sigma, Munich, FRG). Sheets were then mounted in PBS-glycerol and examined by fluorescence microscopy. Ia-positive cells were enumerated in 10× and 40× power fields by using an oculargrid calibrated with a micrometer. All specimens were tested in a blinded fashion and at least 10 separate areas were examined to evaluate the mean number ±SEM of Ia-positive cells/mm^2. White blood cell count and haemoglobin A_{1c} were determined using standard methods.

Statistical analysis

Results were expressed as means ±SEM. Comparisons were made on the basis of the two-tailed Student's t-test. Correlations between LC, monocytes, and insulin were calculated using linear regression analysis.

Results

In healthy control subjects a mean density of 611 ± 33 Ia-positive Langerhans cells/mm^2 of epidermis was obtained (Fig.1). The lowest LC count observed in

normal control subjects was 414 cells/mm^2. Sex and age had no detectable influence on the number of LC (women $n=9$: 633 ± 46 cells/mm^2, men $n=8$: 587 ± 49 cells/mm^2, no positive correlation with age). In the total group of Type 1 diabetic patients, the mean number of LC cells/mm^2 was reduced compared to normal control subjects (480 ± 31 cells/mm^2, $p<0.02$).

Taking duration of diabetes into account, patients with very recent onset of diabetes (T_0) exhibited the lowest LC numbers in comparison to normal control subjects as well as to diabetic patients with a diabetes duration of 6 months (LC/mm^2 T_0: 401 ± 30 vs T_6: 559 ± 43, $p<0.01$, T_0 vs control subjects: 611 ± 33, $p<0.002$, Fig.1). In contrast, out of the 10 patients biopsied 6 months after the onset of diabetes, only one was found to have a clearly diminished epidermal LC count, which was below the lowest normal value (Fig.1). Many of the epidermal samples from patients with Type 1 diabetes showed an irregular staining pattern reflecting either an irregular distribution of LC in their skin or an irregular expression of Ia-antigens.
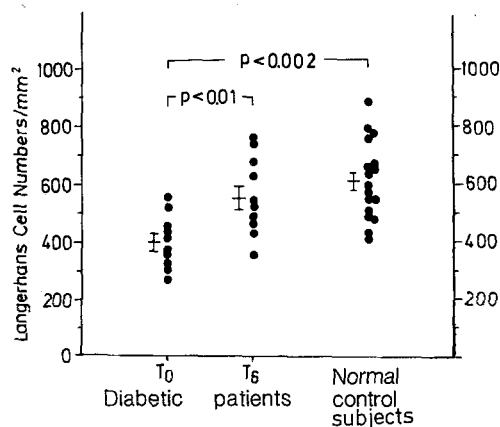

Fig.1. Numbers of epidermal Langerhans cells (mean ±SEM), stained for Ia-antigen, in patients with Type 1 (insulin-dependent) diabetes and normal control subjects. (T_0=diabetes duration of 13 ± 4 days, T_6=diabetes duration of 6 months)
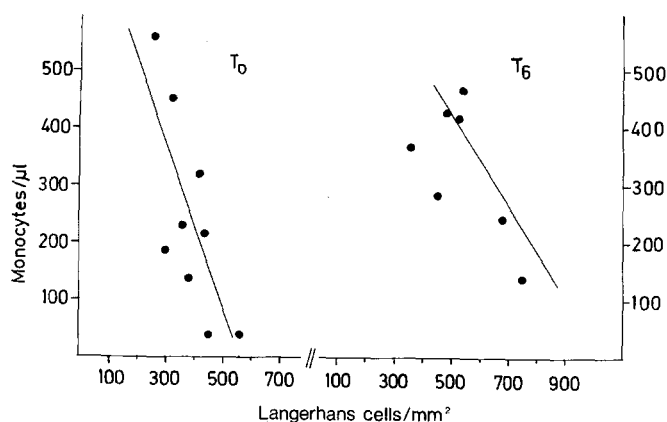

Fig.2. Correlation between epidermal Langerhans cells and blood monocytes in Type 1 diabetic patients (T_0=diabetes duration of 13 ± 4 days; $r=-0.73$, $p<0.01$; T_6=diabetes duration of 6 months; $r=-0.61$, $p<0.05$)

There was no correlation between the number of LC and HbA_{1c} levels. However, the LC counts 6 months after onset of diabetes correlated positively with the daily insulin dose ($r = 0.76$; $p < 0.01$), whereas no such correlation was observed in the recent onset group. There was a tendency to higher blood monocyte levels in diabetic patients in comparison to normal control subjects (241 ± 56 cells/μl at T_0 vs 344 ± 37 at T_6 vs 191 ± 31 in control subjects, $p < 0.05$ for T_6 vs control subjects) and there was an inverse correlation between the number of dendritic cells in the skin and the counts of monocytes in the peripheral blood in patients with newly diagnosed Type 1 diabetes as well as in patients with a diabetes duration of 6 months (Fig. 2).

Discussion

The Langerhans cell is a dendritic immunocompetent cell which resides in the epidermis, but is capable of binding various allergens and migrating to the lymph vessels and local draining nodes to present antigen to T-cells [9].

The present study has demonstrated that Ia-positive Langerhans cells were either generally reduced or even nearly absent in some areas of skin biopsies from Type 1 diabetic patients in the early stage of the disease. The reason for this decrease is presently unknown. None of the patients had been subjected to excessive sun exposure, particularly on the inner aspect of the upper arm, where the biopsies were taken, and none had been treated with steroids. Whether the decrease in the LC density was due to an alteration in Ia-surface antigen or to an actual change in the number of LC remains, however, an open question at the present time. This might be evaluated further by methods for LC-labelling using antibodies against the T_6-antigen, ATPase staining, or electron microscopy [2]. In cutaneous lupus erythematodes, it has been reported that LC could still be detected in the epidermis by electron microscopy, but appeared to be damaged, while HLA-DR, T_6-antigen and ATPase staining were negative [3]. In progressive sclerosis, however, morphologically normal Langerhans cells were seen by electron microscopy, yet they were clearly reduced in number [4].

Provided that there is a loss of LC in newly diagnosed Type 1 diabetic patients, two hypotheses should be taken into consideration. (1) The sequestration of LC from skin to distant areas, e.g. regional lymph nodes or other sites of inflammation. Interestingly, during skin sensitisation reactions of the delayed hypersensitivity type in animal strains, LC were found to be transiently absent from the epidermis and migrate to the regional node presenting antigen to helper T-lymphocytes [9]. (2) A decreased ability of LC to repopulate the epidermis. There are studies indicating that LC are derived from and are continuously repopulated by a mobile pool of precursor cells, which originate in the bone marrow. As LC show similar if not identical surface marker characteristics to cells of the monocyte macrophage series, the most likely precursor cells were considered to be monocytes [2]. We found that the number of circulating monocytes was marginally higher in Type 1 diabetic patients than in normal contol subjects and that there was an inverse correlation between peripheral blood monocyte counts and the number of LC in the skin. This might rather indicate an increased production or release of precursors from bone marrow substituting for the loss of LC in the skin.

In all, the exact mechanism of the alteration in LC density and their functioning in Type 1 diabetes is presently unclear, but there are reports with respect to other autoimmune diseases, which are consistent with our findings [3, 4]. It seems also noteworthy that the diabetic patients 6 months after diagnosis exhibited similar mean Langerhans cell numbers as normal control subjects. This finding might not be surprising, since other immune markers (e.g. T-cell activation and islet cell antibodies) also show a decline from diagnosis to a later stage of the disease [1]. There was no correlation between HbA_{1c} levels and LC counts at any time point of the examination and all patients had fairly controlled glucose values at the time of biopsy. Therefore, a major influence of metabolic control on the results seems to be unlikely.

Finally, it was interesting that there was a correlation of daily insulin dose with LC counts in the 6 months patient group. Although this could be a coincidental finding, it should be mentioned that during the last two years an increasing number of studies have indicated a possible immunosuppressive effect of insulin. It has been shown, e.g. that Type 1 patients treated aggressively with insulin have higher C-peptide levels after one year of onset compared to conventionally treated diabetic patients – similarly to patients treated with steroids and immuran [10].

In summary, these abnormalities in number and distribution of immunocompetent epidermal Langerhans cells seem to indicate a new aspect involved in the pathogenesis of Type 1 diabetes mellitus; and might suggest – 100 years after the death of Paul Langerhans in 1888 – a presently unknown link between Langerhans cells of the skin and the autoimmune-mediated destruction of the pancreatic islets of Langerhans.

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