Photopheresis at onset of type 1 diabetes: a randomised, double blind, placebo controlled trial

J Ludvigsson, U Samuelsson, J Ernerudh, C Johansson, L Stenhammar, G Berlin

Abstract

***Background*—In recent years photopheresis, an extracorporeal form of photochemotherapy using psoralen and ultraviolet A irradiation of leucocytes, has been claimed to be an effective form of immunomodulation.**

***Aim*—To evaluate its effect in type 1 diabetes we performed a double blind, controlled study using placebo tablets and sham pheresis in the control group.**

***Methods*—A total of 49 children, aged 10–18 years of age at diagnosis of type 1 diabetes were included; 40 fulfilled the study and were followed for three years (19 received active treatment with photopheresis and 21 placebo treatment).**

***Results*—The actively treated children secreted significantly more C peptide in urine during follow up than control children. C peptide values in serum showed corresponding differences between the two groups. The insulin dose/kg body weight needed to achieve satisfactory HbA1c values was always lower in the photopheresis group; there was no difference between the groups regarding HbA1c values during follow up. The treatment was well accepted except for nausea (n = 3) and urticaria (n = 1) in the actively treated group. There were no differences regarding weight or height, or episodes of infection between the two groups during follow up.**

***Conclusion*—Photopheresis does have an effect in addition to its possible placebo effect, shown as a weak but significant effect on the disease process at the onset of type 1 diabetes, an effect still noted after three years of follow up.**

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Keywords: photopheresis; diabetes; photochemotherapy; placebo

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In 1983 it was shown that plasma exchange seemed to preserve β cell function at the onset of type 1 diabetes[1]; cyclosporin was subsequently shown to influence the immune mediated process causing type 1 diabetes.[2 3] The cyclosporin effect was limited and transient, and the treatment was therefore regarded as unjustified because the side effects and risks were too serious in comparison with the positive effects.[4 5] Over the years several other immune interventions have been tried, such as azathioprine,[6] CD4 antibodies,[7] immunoglobulins,[8] and linomide,[9] but with minor and transient effects.

In recent years photopheresis has been claimed to be an effective form of immunomodulation.[10] This method was originally used in the treatment of cutaneous T cell lymphoma,[11] but has also been used in various autoimmune diseases with positive results.[12–16] It has also been proposed to be effective in graft versus host disease,[17 18] and to prevent rejection in cardiac transplantation.[19 20] Photopheresis is an extracorporeal form of photochemotherapy using psoralen and ultraviolet A (UVA) radiation. How photopheresis exerts its effects is not fully understood. When nucleated cells, such as lymphocytes, are irradiated with UVA light (wavelength 320–390 nm) in the presence of psoralen, this substance will bind to and intercalate with DNA base pairs, leading to an antiproliferative effect.[21 22] However, this alone cannot explain an immunomodulatory effect of photopheresis. When irradiated cells are reinfused, an immune response could be triggered through UVA and psoralen induced alteration of surface bound molecules, especially affecting activated cells like autoreactive T cells.[10 23 24] Repeated photopheresis may have a booster effect. Thus, the transfusion of transformed cells may be regarded as a kind of vaccination leading to an anti-idiotypic response directed against the transformed, but also against similar pathogenic clones in vivo.[25 26] Some studies in animals support this hypothesis.[27 28]

To our knowledge, treatment with photopheresis has never been evaluated in a randomised, double blind manner and controlled with sham pheresis. Therefore it cannot be excluded that some effects seen after photopheresis in other studies could be attributed to the placebo effect which might be strong after such an invasive intervention. We therefore decided to test photopheresis in type 1 diabetes. This is an immune mediated disease with defined diagnosis and reasonably homogeneous course, in contrast to some other studied diseases such as rheumatoid arthritis and systemic lupus erythematosus which have periods with less symptoms or clinical signs mixed with periods of more severe symptoms or clinical signs. To evaluate the effect of photopheresis we performed a prospective, double blind, placebo controlled study using placebo tablets and sham pheresis in the control group, and we now report on the efficacy and side effects observed during a three year follow up period.

Methods

Three paediatric departments (Linköping, Norrköping, and Jönköping) in the southeast region of Sweden participated in the study. A

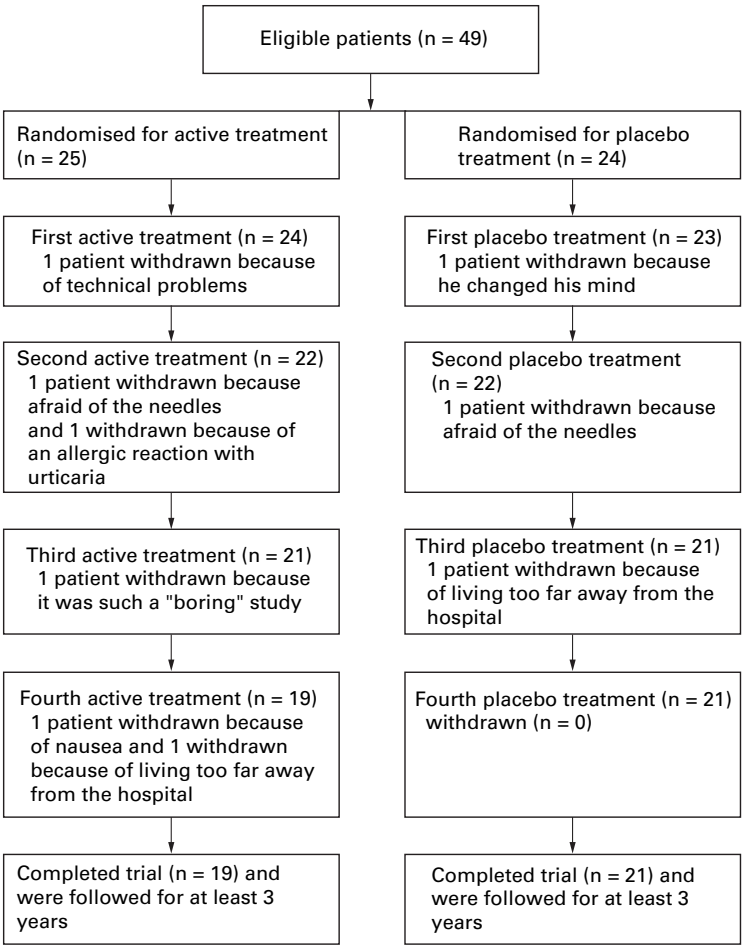

Figure 1 Trial profile. Each square represents an apheresis procedure (active photopheresis or sham procedure) administered on two consecutive days (double treatment).

blood sample was taken at diagnosis, before the first insulin injection. The patients were subsequently treated with intravenous insulin infusion until blood glucose was stable at normal concentrations for at least 24 hours, and the patients were free from ketonuria and had normal acid–base balance. To be eligible the patients had to be 10–18 years of age. The patients and their parents were given careful oral and written information about the study, in compliance with the guidelines of the Research Ethics Committee. Patients who participated in the study received traditional treatment with multiple insulin therapy, diet, regular exercise, and self control and were then randomly (by envelope) allocated either to active treatment or to placebo treatment with sham procedure. The result of the randomisation was blind not only to the patients and their parents, but also to the clinicians and the staff of the hospital departments in charge of the patients during the treatment and follow up period. Only the staff of the apheresis unit knew if a patient was actively treated or not. The randomisation code was not broken until all patients had been followed for at least two years.

The number of patients required for the efficacy analysis was based on a significance level of 5% and a power of 80% to detect an improvement of fasting C peptide or a maximal C peptide response greater than 50%. The estimates of baseline values and variance of C peptide values in children under 15 years of age at diagnosis were based on our earlier studies.[29–31] From these calculations we concluded that 40 patients would be required.

A total of 49 children were included after obtaining informed consent from the children and their parents (fig 1). Nine children, three from the placebo group and six from the actively treated group, withdrew from the study after 0–5 photopheresis procedures. One boy in the control group changed his mind before the first treatment and one child in the active group declined after technical problems with venous access at the start of the first treatment. One child withdrew after two treatments because of an allergic reaction with urticaria after intake of tablets and another withdrew after five treatments because of nausea; both children were in the actively treated group. Two other children withdrew after the first treatment period, one from each group, as they were afraid of the needles; one child withdrew after four treatments because it was such a "boring" study. Finally, two children dropped out, one after two treatments (placebo group) and the other after five treatments, as they lived far away from the hospital and did not want to travel (fig 1). The remaining 40 patients fulfilled the study protocol and were then followed for at least three years with repeated tests of β cell function, clinical parameters, and blood samples for further studies of both humoral and cell mediated immune response.

PHOTOPHERESIS

Photopheresis was performed at the university hospital in Linköping using a UVAR instrument (Therakos, West Chester, Pennsylvania). Patients randomised to active treatment received oral 8-methoxypsoralen (8-MOP; Puvamet, Tika, Lund, Sweden). One hour later, apheresis was started; 240 ml buffy coat and 300 ml plasma were removed by a discontinuous apheresis procedure and diluted with approximately 200 ml saline (final haematocrit of the cell solution was 2–8%). The cell solution was passed as a 1 mm thick film through a disposable transparent plastic channel, exposed to UVA light (2 J/cm^2) for 90 minutes, and returned to the patient's circulation. Peripheral venous access (cubital vein) was used for all treatments.

Because of a variability of psoralen bioavailability after peroral intake, 8-MOP concentration was assessed with the standard dose of 0.6 mg/kg body weight before the start of the first photopheresis treatment. If needed, the individual dosage was adjusted to achieve a peak 8-MOP plasma concentration of >100 ng/ml at the start of apheresis and a concentration of >50 ng/ml in the cell solution. 8-MOP concentration was determined by a standard high pressure liquid chromatography technique.[32] Mean plasma concentration at the start of apheresis was 324 ng/ml (range 50–878) and mean 8-MOP concentration of the cell solution during irradiation was 105 ng/ml (range 10–273).

Table 1 Actual time periods from onset of disease to treatment 1 and time periods between the treatments

	Treatment 1		*Treatment 2*		*Treatment 3*		*Treatment 4*		*Treatment 5*	
	Range (days)	*Mean (days)*	*Range (weeks)*	*Mean (weeks)*	*Range (weeks)*	*Mean (weeks)*	*Range (weeks)*	*Mean (weeks)*	*Range (weeks)*	*Mean (weeks)*
Active	5–20	11.3 (3.9)	2–6	3.6 (0.9)	4–8	5.7 (0.9)	9–12	9.9 (1.3)	12–18	13.8 (1.5)
Placebo	3–17	10.0 (3.8)	2–5	3.4 (0.7)	5–8	5.7 (1.0)	8–11	9.5 (0.9)	12–17	13.5 (1.3)

Results expressed as mean (SD).

Patients randomised to the control group received placebo tablets. The procedure at the apheresis unit was similar in the actively treated and control patients. All patients put their arm through a curtain and were venepunctured. Neither the patients nor their parents could see the apheresis instrument or the disposables. Blood was taken for analysis from all patients. In the active treatment group, photopheresis was then performed as described above. Control patients received a saline infusion at a very slow flow rate. The UVAR instrument was turned on and the centrifuge was working at regular intervals, mimicking a photopheresis procedure, but no blood was processed in the instrument. The staff attempted to behave in a similar way to all patients. No staff from the Department of Pediatrics were allowed at the apheresis unit during treatment.

The apheresis procedure (active photopheresis or sham procedure) was administered on two consecutive days (double treatment). The aim was that the first double treatment should be given at day 5–6 after diagnosis and then repeated after two, four, eight, and 12 weeks so that every patient should receive five double treatments in a three month period. In reality this time schedule was not strictly adhered to, due to school and social commitments (table 1). Blood glucose values were measured before and after each treatment; haemoglobin and white blood cells, as well as fibrinogen, antithrombin, prothrombin, and activated partial thromboplastin time (APTT) were determined only before each treatment. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and creatinine were measured the first day of each double treatment session.

Table 2 Background variables in actively treated (photopheresis) and placebo treated (sham pheresis) groups

	Active group	*Placebo group*
Age at diagnosis	13.8 (1.6)	13.4 (1.9)
Weight at diagnosis (kg)	49.7 (12.3)	45.6 (9.9)
Height at diagnosis (cm)	163.8 (11.6)	160.3 (11.8)
Duration of symptoms before diagnosis (days)	24.7 (21.6)	16.6 (14.2)
Blood glucose at diagnosis (mmol/l)	23.9 (7.2)	23.4 (8.1)
pH at diagnosis	7.30 (0.1)	7.36 (0.1)
Base excess at diagnosis	4.0 (3.6)‡	3.8 (7.3)‡
Weight loss prior diagnosis (kg)	5.8 (4.5)*	2.7 (1.6)*
HbA1c at diagnosis (%)	10.3 (2.5)	9.6 (2.7)
Number of hours to obtain stable blood glucose values	9.2 (4.8)	9.0 (8.1)
Infection 2 months before diagnosis (%)	53	30
Pronounced ketonuria at diagnosis (%)	65†	29†
Allergic disease in the child (%)	18	24
Boys (%)	68	57
Type 1 diabetes in the family	16	19
DR3/4 (%)	86	87

Results expressed as mean (SD).
*$p < 0.02$, †$p < 0.05$, ‡$p = 0.1$.

ASSAYS

C peptide in blood was measured according to the method of Heding,[33] in the fasting state and after a standardised breakfast meal,[29 30] and in urine collected overnight.

Islet cell antibodies (ICA) were detected by immunofluorescence on human pancreas sections according to Bottazzo and colleagues.[34] Our laboratory participated in international ICA workshops during 1993–1996, in which we reached a specificity and sensitivity of 100%.

Antibodies to glutamic acid decarboxylase (GAD) were measured according to Grubin and colleagues.[35] Our laboratory participated in the second GAD antibodies proficiency test in 1996 and reached a sensitivity and specificity of 100%. Validity and consistency were also 100%.

STATISTICAL ANALYSIS

The results were analysed by χ^2 or two tailed Student's *t* tests; when there was indication of skewed distribution, the Mann–Whitney U test was used. Differences were considered significant at $p < 0.05$. Results are expressed as mean (SD) or mean with 95% confidence interval (95% CI). Area under curve (AUC) was calculated according to Matthews and colleagues.[36]

ETHICS

Placebo treatment is acceptable if there is no existing effective treatment. This is the case at onset of type 1 diabetes as no method to save β cell function has been accepted for routine clinical use. Although sham pheresis could be regarded as unethical, especially when used in children and teenagers, photopheresis is a very distinct intervention by which a placebo effect could be expected. Such a placebo effect might influence the general attitude to diabetes treatment, which may improve compliance of diabetes treatment, leading to better metabolic control; this in turn might influence residual β cell function. We therefore foresaw a risk that an open study could lead to false positive results with long term negative consequences for many patients treated with photopheresis in vain in the future. Thus, the Research Ethics Committee agreed unanimously that the most ethical way to do the study would be with a double blind design, with controls treated with placebo tablets and sham pheresis.

Results

BACKGROUND PARAMETERS

After withdrawals there were 19 children in the active group and 21 in the placebo group (fig 1). Table 2 shows that there were no significant

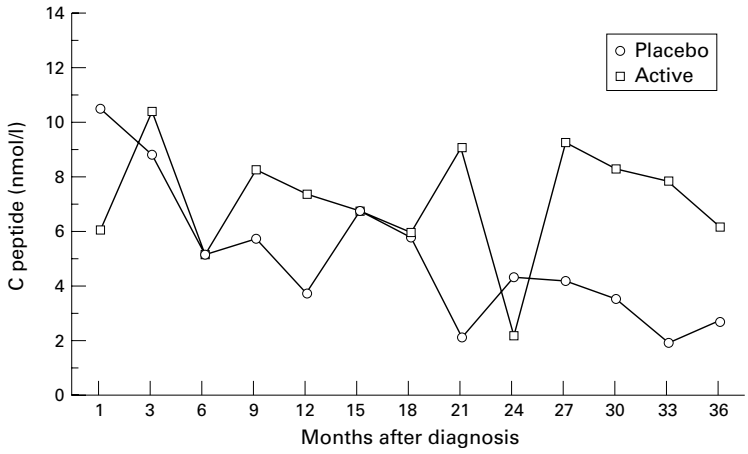

Figure 2 Mean C peptide values in overnight urine.

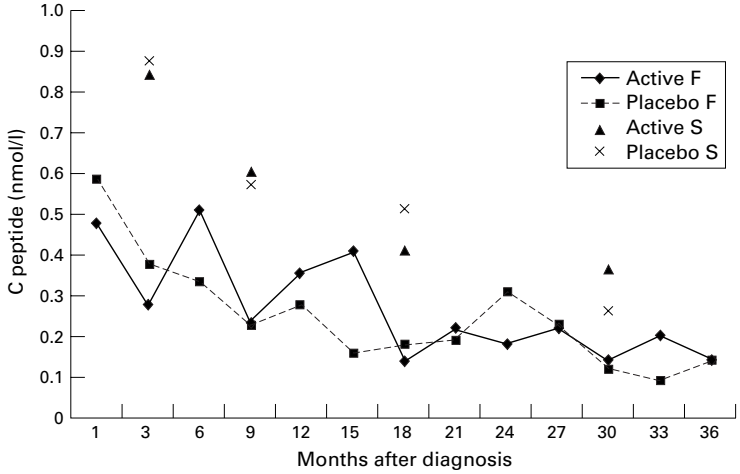

Figure 3 Mean fasting (F) and breakfast stimulated (S) serum C peptide values.

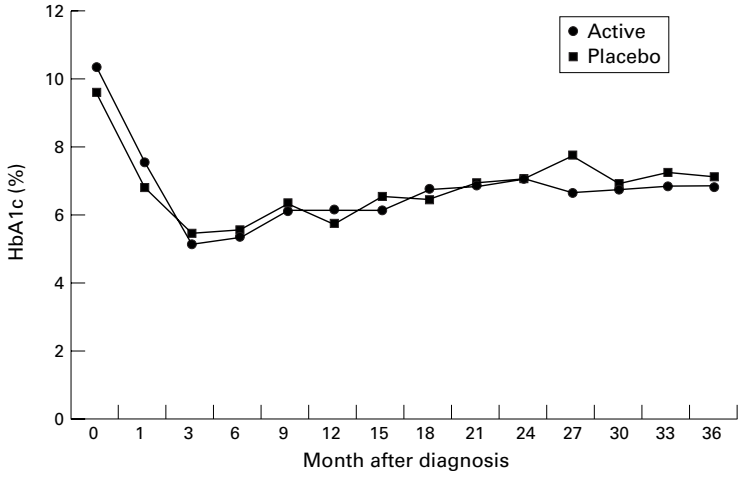

Figure 4 HbA1c values.

differences in age, weight, height, or blood glucose values at onset. However, despite random allocation to active or placebo treatment, children in the active group seemed to have a more serious disease, manifested as greater weight loss before diagnosis ($p < 0.02$), a tendency to lower base excess ($p = 0.1$), and pronounced ketonuria (++ or +++) more often at diagnosis ($p < 0.05$). Children in the active group also had longer duration of symptoms before diagnosis, higher HbA1c values at diagnosis, and lower pH than children in the placebo group (all NS).

Slightly more boys were randomly allocated to the active group (13/19; 68%) than to the placebo group (12/21; 57%) (table 2). There were no differences regarding type 1 diabetes within the family, allergy, and HLA DR3/4 (table 2). Fifteen of 16 tested children in the active group were ICA positive compared with 14 of 15 tested in the placebo group. GAD antibodies were as common in the active group (11 of 14 tested children) as in the placebo group (10 of 17 tested children).

C PEPTIDE VALUES DURING FOLLOW UP

C peptide values in serum (fasting) and urine (overnight) were measured at month 1, 3, 6, and 9, and then every third month up to month 36. At 3, 9, 18, and 30 months the children were tested with a standardised breakfast load and maximal serum C peptide was measured. Figure 2 shows that the actively treated group had higher C peptide concentrations in the urine during the follow up period in comparison with the control group. At month 1, before the end of photopheresis treatment, values in the placebo group were significantly higher ($p = 0.04$); this tended to be reversed during follow up, especially in months 21, 30, and 33 ($p = 0.1$). AUC from month 1 to month 36 for the actively treated group was 250.9 nmol/l compared with 166.2 nmol/l for the placebo group. Fasting serum C peptide values showed corresponding differences between the two groups; there were no differences regarding maximal C peptide values in serum (fig 3). On two occasions there was a tendency for higher fasting serum values in the actively treated group: at month 6 ($p = 0.06$) and month 15 ($p < 0.03$). No differences were seen for stimulated C peptide values.

INSULIN DOSES AND HbA1c VALUES DURING FOLLOW UP

Insulin doses and HbA1c values were measured at the same time intervals as C peptide. There was no difference between the groups regarding HbA1c values (fig 4); the proportion of children with HbA1c <6% was the same in the two groups during follow up period. However, insulin doses/kg body weight needed to achieve stable blood glucose values were, with the exception of month 1 (before the end of the photopheresis treatment), always lower in the active group (fig 5). At months 18, 21, and 33 this difference was significant ($p < 0.02$) as well as at months 9 and 15 ($p < 0.04$); at month 12 there was a tendency towards significance ($p = 0.1$). AUC was also lower in the active group (23.6 units) compared with the placebo treated group (32.4 units). The proportion of children with an insulin dose/kg <0.5 U was, with exception of months 1 and 3, always higher in the actively treated group, although the difference never reached significance; there was a tendency towards significance at months 15, 18, and 30 ($p = 0.1$).

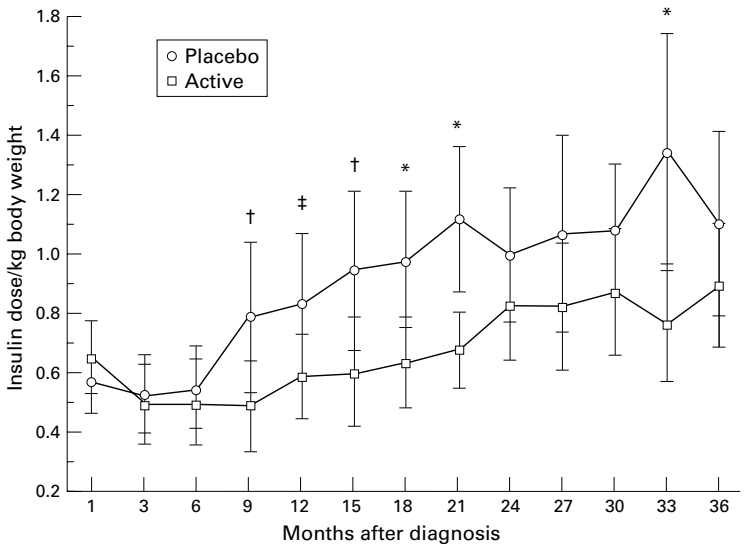

*Figure 5 Mean (95% CI) insulin doses/kg body weight needed to keep a stable blood glucose value. *p < 0.02; †p < 0.04; ‡p < 0.1.*

During follow up, two boys (one in each group) went into complete remission for three to six months. The boy in the active group seemed to have a more severe manifestation at onset as in addition to a lower pH, lower C peptide concentration in serum, and higher HbA1c value at diagnosis, a more pronounced weight loss before diagnosis. At diagnosis he reached a stable blood glucose after 12 hours, compared with 2.5 hours for the boy in the placebo group.

ADVERSE EFFECTS OF PHOTOPHERESIS

One patient withdrew from the study because of nausea, and another because of urticaria. Two other children, also in the actively treated group, experienced a short period of nausea in connection with one of the treatments. Otherwise the treatment was well accepted.

We found no difference in blood glucose, haemoglobin, or leucocyte count between the two patient groups in connection with photopheresis. On several occasions the number of white blood cells decreased somewhat during the treatment; this occurred in both the active and the placebo group. There were no differences in fibrinogen, thrombin, APTT, creatinine, ALT, and AST between the groups. Photopheresis had no effect on weight or height during follow up. With regard to infections during follow up, 18/19 patients in the actively treated group had 43 episodes of common cold (11 with fever), seven of gastroenteritis, seven of tonsillitis, and four of unspecified fever. In the placebo group, 17/21 patients had 41 episodes of common cold (11 with fever), three of gastroenteritis, six of tonsillitis, and four of unspecified fever. One child in this group had varicella.

Discussion

An important question is whether it is ethically justified to let patients, especially children, receive placebo treatment in the form of sham pheresis. We found the study design justified this as there exists no effective intervention in type 1 diabetes, and a placebo controlled study is the only way to discern the possible treatment effect from the plausible placebo effect. The Ethics Committee accepted this.

Photopheresis is believed to modulate the immune process. As concluded from other immune intervention studies in type 1 diabetes,[3 6 8 9] one can expect that a maximum of 40–50% of newly diagnosed diabetic children have an ongoing β cell destructive process, even though the majority of them have autoantibodies. This means that if photopheresis is effective one should not expect efficacy in more than a small proportion of patients. In fact we noticed an effect of photopheresis as the β cell function, reflected by C peptide secretion in urine and serum, was slightly better, and the insulin requirement was significantly lower in the actively treated group compared with the placebo group. At the same time the HbA1c values were at least not lower in the control group but rather the opposite, especially at the end of the follow up period. Thus all parameters point in the same direction. This result is even more convincing as the actively treated group by chance happened to be more seriously ill at onset with more weight loss, more pronounced ketonuria, higher HbA1c values, and lower pH, all parameters known to be related to low β cell function.[37] Thus, in this double blind, placebo controlled study we have been able to show that photopheresis has an effect on the process leading to diabetes and this effect is seen still after three years follow up. However, the clinical effect is really very weak compared to some other immune interventions,[2 3 6 8 9] and further studies are needed to determine how and when photopheresis should be used. In the recently published study to prevent rejection of heart transplantation,[19] the authors used a combination of cyclosporine, azathioprine, and prednisone together with photopheresis. This can probably not be justified in the treatment of diabetes, especially not in children and teenagers, because of the potential serious side effects, but one can speculate in other types of combination treatments. Furthermore, we do not know if the timing we used was optimal, or whether photopheresis should be continued with increasing intervals after the initial three months from diagnosis. At the time when our trial was planned most photopheresis studies used protocols with double treatment once a month for at least three months. In recent years there has been a trend towards more frequent treatments at the start and also longer treatment periods. We chose a three month treatment period for practical and economic reasons. In comparison with other trials we believe that the placebo controlled design of our study strengthened the possibility of showing a positive effect of photopheresis. However, it cannot be ruled out that more frequent treatments over a longer period might have improved the result.

We conclude that photopheresis does have an effect in addition to its possible placebo effect. A tempting possibility would be to use photopheresis for prevention of diabetes in

high risk groups as defined by autoantibodies and genetic markers. However, the effect on the disease process at the onset of type 1 diabetes is very weak and more knowledge is needed before it can be considered as a treatment modality of newly diagnosed type 1 diabetes. An advantage with photopheresis treatment is the low frequency of side effects related to the treatment. On the other hand, the practical efforts and the high treatment cost are obvious disadvantages.

We are very grateful to the children who gladly and with enthusiasm participated in this extensive study. The skill of the staff of the apheresis unit in performing the photopheresis treatments and the sham procedures is acknowledged. We also thank Lena Berglert, Sonja Hellström, and Eva Isaksson for technical and administrative assistance; Iris Franzen for performing all standardised breakfast loads; and Anja Jacobsson for help with the database. This study was supported by Barndiabetesfonden (Swedish Child Diabetes Foundation), Novo Nordisk Foundation, Lundström Foundation, Söderbergs Foundation, Swedish Diabetes Association, JDF-Wallenberg Foundation (K98-99JD-12813-01A), County Council of Östergötland, and Swedish Medical Research Council (K99-72X-11242-05A). The study was also in part sponsored by Therakos Inc. who provided the disposables for the photopheresis treatments, and Tika, Lund, Sweden, who provided the Puvamet tablets.

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