Diabetologia (1987) 30: 66-71

Diabetologia

Originals

Immunology in diabetic pregnancy: activated T cells in diabetic mothers and neonates

U. Di Mario[1], F. Dotta[1], P. Gargiulo[1], J. Sutherland[1], D. Andreani[1], K. Guy[4], A. Pachi[2] and F. Fallucca[3]

[1] Department of Endocrinology 1, [2] III Clinica Ostetrica and [3] Diabetology Unit, University "La Sapienza", Rome, Italy;
[4] MRC Clinical and Population Cytogenetics Unit, Edinburgh, UK.

Summary. Lymphocytes bearing surface antigens indicating early and full activation have been evaluated, in addition to T cell subsets, in blood samples from diabetic pregnant patients, neonates from diabetic mothers and control groups. The type of diabetes and the trimester of pregnancy were taken into account. Monoclonal antibodies were used to enumerate total T cells, helper/inducer, cytotoxic/suppressor T lymphocytes and activated mononuclear cells using antibodies binding lymphocyte surface antigens as markers of early lymphocyte activation, and MHC Class II surface antigens as markers of late activation. A decrease in T-helper cells during the third trimester of pregnancy in Type 1 (insulin-dependent) and gestational diabetic patiens ($p < 0.02$) and a decrease in T-suppressor cells in Type 2 (non-insulin-dependent) diabetic pregnant patients during the third trimester ($p < 0.01$) were observed in relation to normal values. As in normal pregnancy, 4F2-positive cells were increased in 48% of diabetic pregnant patients during the second and third trimesters of gestation. Class II-positive cells were increased in almost 60% of Type 1 and gestational diabetic patients during the last trimester of pregnancy in comparison with normal pregnant women and control subjects. A decrease in T-helper cells ($p < 0.02$) and a clear increase in 4F2-positive cells ($p < 0.001$) and Class II-positive lymphocytes ($p < 0.005$) were observed in the infants of diabetic mothers in comparison with control subjects. The maternal cellular immune system, actively alerted in pregnancy, is fully activated in a number of Type 1 and gestational diabetic pregnant patients. Activated lymphocytes are even found in the neonates of diabetic mothers, but these do not trigger the events leading to the onset of diabetes in the short term.

Key words: Diabetic pregnancy, infants of diabetic mothers, T cells, activated lymphocytes.

Pregnancy is one of the most fascinating immunological models for studying the interaction of diverse immune components in the recognition and tolerance of an antigenically different organism. Despite the importance of immunological aspects in pregnancy, very little is known even about basic immune parameters in normal pregnancy; contradictory findings have been reported in different studies [1, 2]. In particular, controversial data are available on the phenotyping of T cell subsets, whereas information is scarce regarding the possible expression of surface antigens on activated T cells both during pregnancy and in neonates [1-2].

Diabetes mellitus, on the other hand, is characterised by several immune abnormalities related to pathogenetic events, metabolic derangement and insulin treatment. Among others, an increase in activated T cells and modifications in the helper/suppressor ratio have been found in newly diagnosed Type 1 diabetic patients, whereas a decrease in T cells with helper phenotype together with a increase in T cells with suppressor cytotoxic phenotype has been observed in long standing Type 1 diabetes [3-4].

In diabetic pregnancy, the immunological abnormalities occurring in diabetes are superimposed on the background immunological modifications characteristic of pregnancy itself and may influence the course and outcome of pregnancy and the development of the foetus. In addition, the occurrence of gestational diabetes provides us with the opportunity to study subjects during the very first stages of the development of overt diabetes; some of these subjects may be potential Type 1 diabetic patients during the pre-clinical latency period [5].

Despite the scientific interest, very few studies have so far been reported on the cellular immune modifications of diabetic pregnancy and on the neonates of diabetic mothers [3].

In this study T cell subsets and activated T cells have been evaluated in Type 1 and Type 2 diabetic mothers, gestational diabetic patients, normal pregnant women, in a number of their respective neonates and in normal women. A panel of monoclonal antibodies against different T cell surface antigens has been used to evaluate lymphocytes in different stages of activation.

Table 1. Clinical and metabolic data of subjects included in the study

Subjects	Type 1 pregnant	Type 2 diabetic	Gestational patients	Normal pregnant women	Normal control subjects
Age (years)	28.0± 4.0	33.6± 5.0	33.0± 4.7	28.0± 2.0	29.0± 4.0
Duration of diabetes (years)	12.3± 5.7	8.5± 5.7			
Duration of gestation (weeks)	38.0± 1.5	37.7± 1.5	39.3± 1.00	40.0± 1.3	
C peptide levels (pmol/l)	148 ±45	698 ±94	1368 ±180	629 ±140	645 ±112
Insulin requirements (U/day)	76.0±16	62.0±13	30.0± 12		
HbA_{1c} (%)	6.0± 0.6	6.2± 0.2	6.8± 0.9	5.2± 0.3	5.8± 0.2
Percentage of patients body mass index (%)					
<80th percentile	28.6	-	-	3.4	
80th–100th percentile	52.3	12.5	36.4	70.0	
101st–120th percentile	14.3	87.5	45.4	22.2	
>120th percentile	4.8	-	18.2	4.4	

Subjects and methods

Patients

Seventy-four samples from diabetic pregnant patients (38 Type 1, 19 Type 2 and 17 gestational diabetic patients; 27 during the first trimester, 19 during the second and 28 during the third trimester of pregnancy) attending the Obstetric Clinic, Rome University, were included in the study. The type of diabetes was stated according to the following criteria: (a) presence of diabetes before pregnancy or impaired oral glucose tolerance during pregnancy; (b) need of insulin and/or ketosis tendency before pregnancy; (c) residual B-cell function based on circulating C-peptide levels. Since patients entered the study after varying lapses of time from diagnosis, the presence of islet cell antibodies and the body mass index were not considered satisfactory criteria to group patients.

Type 1 diabetic pregnant patients were all ketosis-prone and insulin-dependent before pregnancy and all had circulating C-peptide levels below 200 pmol/l. Type 2 diabetic pregnant patients all suffered from overt diabetes mellitus before pregnancy, were not ketosis-prone, had circulating C-peptide levels above 200 pmol/l and were treated with oral hypoglycaemic agents or diet before pregnancy. Those women who presented an impaired oral glucose tolerance test during pregnancy were classified as gestational diabetic patients. Clinical and metabolic data concerning the patients included in our study are shown in Table 1.

All patients were treated with human insulin during pregnancy. Toxaemia occurred in 7% of patients during the third trimester of pregnancy. The incidence of Caesarian section was 39% (60% in Type 1, 25% in Type 2 and 35% in gestational diabetic patients).

Forty neonates were subjected to clinical examination. Eleven cord blood samples from neonates of the above diabetic mothers were also included in the immunological study. Their mean neonatal birth weight was 3306±632 g. Eighteen percent of neonates were overweight, 7% presented neonatal hypoglycaemia and 13% revealed hyperbilirubinaemia at birth. Of the infants subjected to clinical examination, one infant of a Type 1 diabetic mother died in the womb and one of a Type 2 diabetic mother died after birth.

Sixty-three normal pregnant women were included in the study, together with 18 normal neonates. Metabolic data are given in Table 1. The mean neonatal birth weight was 3620±350 g.

Twenty-six adult healthy women were included in the study to act as normal control subjects. Metabolic data are also shown in Table 1.

Samples were collected at the following stages of gestation: trimester I, 12±2 weeks; trimester II, 24±1.3 weeks; trimester III, 36±2 weeks

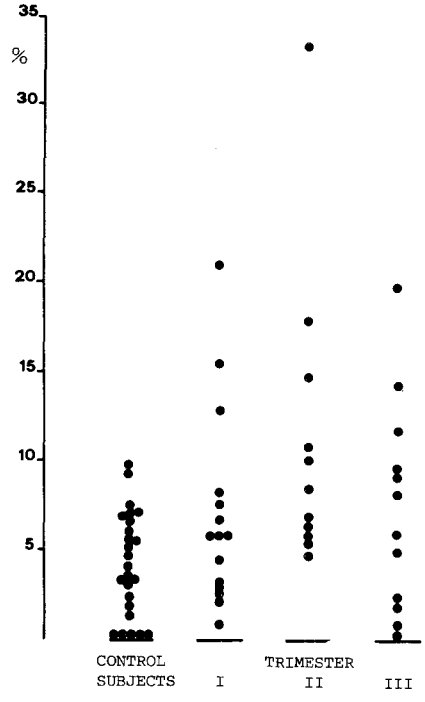

Fig. 1. 4F2-positive cells in Type 1 diabetic pregnant patients

T cell subpopulations

T cell subsets were evaluated using OKT3, OKT4 and OKT8 antibodies (Ortho Diagnostics, Raritan, NJ, USA) to reveal the total T cell population, the helper/inducer and the suppressor/cytotoxic T cell subsets respectively.

Cell surface antigens were measured using an indirect immunofluorescence technique. A sample of 200 cells were counted using a Hamex chamber, which allows the use of very low initial concentrations of cells [6], and a Leitz diavert fluorescence microscope with filters for fluorescein. The same technicians counted all the samples.

Activated T cells

Activated T cells were enumerated using the following monoclonal antibodies: 4F2 (which binds a 120 KD molecular weight glycopro-

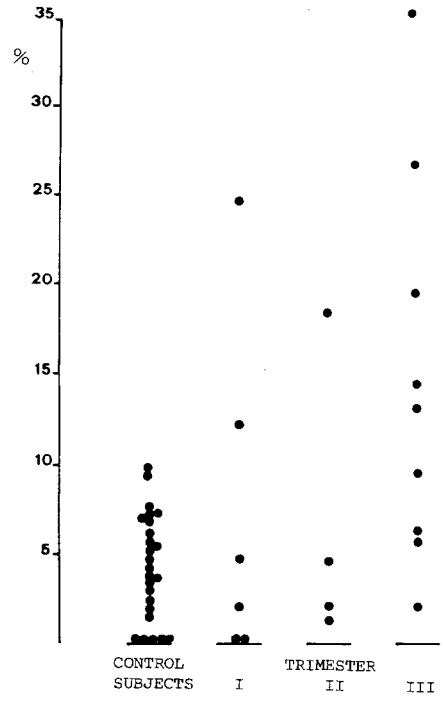

Fig. 2. 4F2-positive cells in Type 2 diabetic pregnant patients

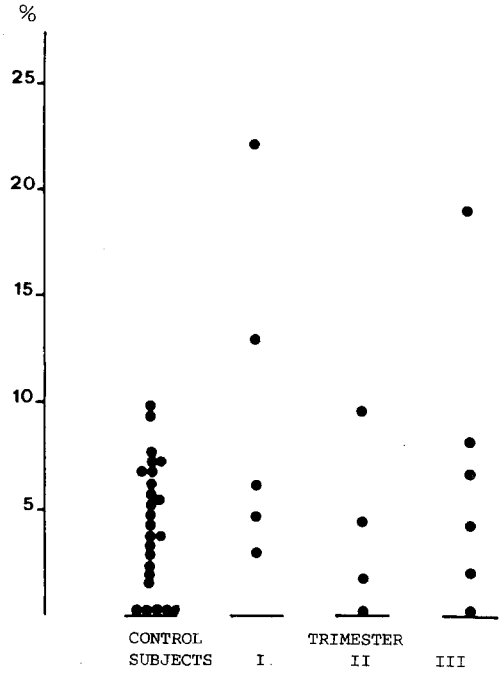

Fig. 3. 4F2-positive cells in gestational diabetic patients

tein present on monocyted and activated B and T cells and is a marker of early activation) [7]; L243 [8], DA6.164 and Da6.231 [9] (which bind different antigenic determinants of the non-polymorphic epitope of the beta-chain of Class II surface antigens). T cells bearing Class II antigens were detected using a double-staining immunofluorescence method developed in our laboratory [10].

After having incubated lymphocytes with one of the Class II antibodies (L243, DA6.164 or DA6.231) and having added fluorescence-labelled antimouse antibody, T cells were killed by adding an anti-pan-T-cell cytotoxic mouse monoclonal antibody (OKT3, Ortho Diagnostics) at a ratio of 0.1 μg per 3×10^5 cells, and freeze-dried rabbit complement. Cells were than incubated at 37 °C for 30 min. After

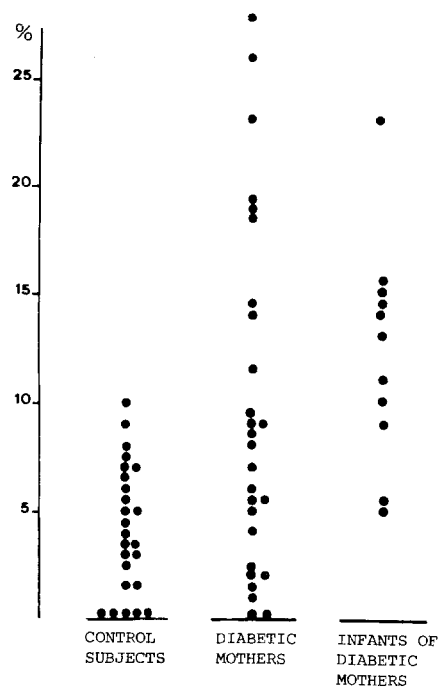

Fig. 4. 4F2-positive cells in diabetic mothers and in their neonates

adding 10 μl of ethidium bromide (50 μg/ml), the cells were washed once and counted. Class II antigen-positive T cells show a red nucleus with a rim of green fluorescence, B cells show the rim of green fluorescence alone and T cells show only the red nucleus.

Since activated T cell values are positively skewed in the normal population, the limit of positivity for this assay was taken as above the 90th percentile (8% for 4F2-positive cells and 2.8% for Class II-positive cells).

Statistical analysis

Student's t-test and Mann-Whitney U-test for unpaired data were used to evaluate the results. A p value of less than 0.05 was considered significant.

Results

Diabetic mothers

Type 1 diabetic patients. The percentage of OKT4-positive cells was significantly decreased in the second and third trimesters of pregnancy ($p < 0.01$ and $p < 0.02$ respectively, Table 2 A) in comparison with control subjects. A significant increase in 4F2-positive cells was found in the second ($p < 0.001$) and third ($p < 0.01$) trimesters of gestation when compared to the normal control group (Fig. 1). Class II-positive T cells were found, with at least one of the monoclonal antibodies, in the second and third trimesters of gestation, respectively, in 60% and in 57.1% of patients studied ($p < 0.01$ vs. normal pregnant women) (Fig. 5). When only the L243 positivity was considered, the percentages were 41% and 38% respectively.

Type 2 diabetic patients. OKT8-positive cells were significantly decreased in the third trimester of pregnancy when compared to normal control subjects, Type 1 diabetic and normal pregnant women ($p < 0.01$, Table 2 B).

A significant increase in 4F2-positive cells was found in the third trimester ($p < 0.01$ vs. normal control subjects, Fig. 2). No statistically significant modification was observed in Class II-positive T cells (Fig. 5).

Gestational diabetic patients. Total T cells were decreased in the third trimester of pregnancy when compared to the control group and to Type 1, Type 2 diabetic and normal pregnant women. OKT4-positive cells were decreased in the third trimester in comparison with Type 2 diabetic pregnant women and with normal control subjects (Table 2 C). An increase in 4F2-positive cells was found in the first trimester of gestation (Fig. 3). Class II-positive T cells were found with at least one of the monoclonal antibodies used in the third trimester of gestation in 60% of patients studied and in 40% when only the L243 positivity was considered ($p < 0.005$ vs. normal pregnant women, Fig. 5).

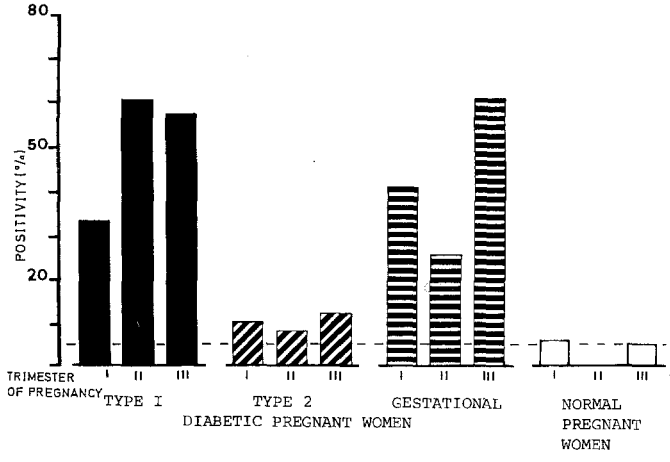

Fig. 5. Class II-positive T cells expressed as percentage of positivity in Type 1, Type 2 and gestational diabetic pregnant patients, and in normal pregnant women

Neonates of diabetic mothers

OKT4-positive cells were significantly decreased in infants of diabetic mothers ($p < 0.02$, Table 3). 4F2-positive cells were increased in neonates of diabetic mothers ($p < 0.001$ vs. normal control subjects, Fig. 4). Class II-positive T cells were found in 55% of neonates of diabetic mothers ($p < 0.005$ vs. normal control subjects) with at least one of the monoclonals, whereas the positivity was 40% when only the L243 positivity was considered. No correlation was found between the presence of class II-positive T cells and neonatal complications and/or labour stress (length of labour, type of delivery, anaesthesia, etc.).

Normal control subjects

Pregnant women. Total T cells were significantly decreased in the first trimester of pregnancy when compared to normal control subjects ($p < 0.05$, Table 2 D). A significant increase in 4F2-positive cells was observed in the second (56%) and third (48%) trimesters of pregnancy ($p < 0.002$) when compared with the control group.

No statistically significant modification was observed in Class II-positive T cells (Fig. 5).

Normal neonates. The percentages of OKT3- and OKT4-positive cells were significantly decreased ($p < 0.02$ and $p < 0.01$ respectively) in comparison to normal control subjects (Table 3). 4F2-positive cells were significantly increased ($p < 0.001$) in comparison to normal control subjects. Class II-positive T cell levels were not statistically different from those observed in the control group (11% vs. 5%).

Non-pregnant adult subjects. The mean and standard deviations, in percentages, of OKT3-, OKT4- and

Table 2. Circulating T cell subsets expressed as percentages of mononuclear cells

	OKT3	OKT4	OKT8	T4/T8
Type 1 diabetic pregnant patients				
I trimester	56.2 (±10.6)	38.4 (± 9.4)	20.6 (±8.3)	2.20 (±1.06)
II trimester	57.0 (± 8.7)	34.6 (± 4.5)[a]	21.3 (±3.5)	1.69 (±0.41)[f]
III trimester	61.4 (± 7.9)	36.4 (± 6.3)[b]	21.6 (±6.7)	1.81 (±0.65)
Type 2 diabetic pregnant patients				
I trimester	56.9 (± 9.0)	40.9 (± 9.0)	15.5 (±5.6)	2.97 (±0.86)
II trimester	56.2 (± 5.6)	40.7 (± 1.0)	18.1 (±5.6)	2.56 (±1.07)
III trimester	58.6 (± 5.5)	44.5 (± 7.5)	14.1 (±4.6)[c]	3.75 (±1.58)[g]
Gestational diabetic women				
I trimester	56.8 (± 4.6)	37.0 (± 9.2)	20.1 (±3.7)	1.87 (±0.51)
II trimester	56.0 (± 4.2)	43.3 (± 2.3)	16.4 (±2.4)	2.70 (±0.48)
III trimester	48.6 (± 8.9)[d]	36.2 (± 6.3)[b]	16.7 (±5.0)	2.46 (±1.44)
Normal pregnant women				
I trimester	52.7 (± 7.8)[e]	37.1 (± 6.5)	17.7 (±6.3)	2.45 (±1.15)
II trimester	57.8 (± 7.0)	39.2 (± 6.9)	18.6 (±3.8)	2.23 (±0.72)
III trimester	57.1 (± 8.9)	39.3 (±10.9)	18.6 (±7.2)	2.65 (±1.68)
Normal control subjects	57.8 (± 7.2)	41.4 (± 7.3)	19.4 (±5.4)	2.32 (±0.65)

[a] $p < 0.01$ vs. B, C, D, E; [b] $p < 0.02$ vs. B, E; [c] $p < 0.01$ vs. A, D, E; [d] $p < 0.02$ vs. A, B, D, E; [e] $p < 0.05$ vs. E; [f] $p < 0.01$ vs. C, E; $p < 0.02$ vs. D; [g] $p < 0.01$ vs. A, E

Table 3. Circulating T cell subsets expressed as percentages of mononuclear cells in neonates of normal and diabetic mothers

	T cell subsets			
	OKT3	OKT4	OKT8	T4/T8
Infants of diabetic mothers	55.1 ± 12.3	34.8 ± 10.5[a]	19.4 ± 5.6	1.89 ± 0.75
Infants of normal mothers	49.6 ± 13.0[a]	34.7 ± 9.9[a]	17.4 ± 7.1	2.35 ± 1.38
Control subjects	57.8 ± 7.2	41.4 ± 7.3	19.4 ± 5.4	2.32 ± 0.65

[a] $p < 0.02$ vs control subjects

OKT8-positive cells are reported in Table 2; means and standard deviations were 4.3 ± 2.8, 1.1 ± 0.9 and 1.2 ± 1.0, respectively, for 4F2 positive cells, 231- and L243-positive T cells.

Discussion

The maternal immune status in diabetic pregnancy may be regarded as the superimposition of diabetic immune abnormalities on the immunological modifications which normally occur during pregnancy. It is of interest to distinguish between these interrelated events in order to evaluate the possible consequences on the course and outcome of pregnancy and the normal development of the foetus.

Diabetic pregnant women may reveal humoral immune abnormalities such as circulating islet cell autoantibodies, insulin antibodies and immune complexes. It has been reported that the first two abnormalities may be transferred to the neonate and, while islet cell antibodies have not been correlated with the development of diabetes in the neonate [5, 11, 12], insulin antibodies have been found to correlate with some neonatal clinical complications [13, 14]. Circulating immune complexes have been correlated with complications during the course of pregnancy [14–16] and are thought to be also deposited or formed in the placenta [17], thus possibly contributing in a number of cases to the development of tissue damage. Even if insulin does not cross the placenta, and insulin antibodies do not induce significant insulin transfer, the passage of maternal insulin antibodies across the placenta may lead to the formation of insulin-anti-insulin complexes [13, 18]. Indeed, these complexes have been found in the circulation of neonates, with characteristics which differ from those of their mothers, and are thought to play a role in neonatal hypoglycaemia [18].

This study underlines the presence of several cellular immune abnormalities in the diabetic pregnant woman. These modifications differ according to the type of diabetes.

Type 1 diabetic pregnant women showed a significant reduction in helper/inducer T cells. This reduction was also found in long standing Type 1 diabetic patients. Thus, the reduction in cells bearing the T4 phenotype seems to be related to the immune abnormalities linked to diabetes rather than to the events occurring in pregnancy.

Type 2 diabetic pregnant women revealed a decrease in the OKT8-positive T cell population. Type 2 diabetic patients included in our study had previously been treated with diet or oral agents; on entering the study at the beginning of pregnancy they were thereafter treated with heterologous insulin. The explanation of the T cell modification is not clear, and the finding may reflect events linked either to Type 2 diabetes of long duration or to metabolic and therapeutic modifications in pregnancy.

A decrease in helper/inducer cells in T cell subsets has been found in gestational diabetic patients in the third trimester of pregnancy and is comparable to that in Type 1 diabetic patients.

The modifications of T cell subpopulations reported above do not seem to reflect adequately the complex and fascinating sequence of immunological events which occurs in pregnancy. The failure of the maternal immune system to respond to the antigenically foreign foeto-placental unit must involve complex immunological mechanisms. Monoclonal antibodies defining T cells during activation and differentiation have therefore been used for further studies.

A clear increase in 4F2-positive cells is present both in normal and diabetic pregnant women and in their infants. It is conceivable that during pregnancy resting T cells are activated or induced to enter the first phase of the cell cycle (G0→G1 transition) and that this coincides with the expression of the 4F2 antigen on the cell surface. Further progression through the cell cycle, leading to mitosis and expression of the Tac antigen, Class II antigens and functional maturity, appears to be blocked. A possible interpretation of the increase in 4F2-positive cells is in line with the concept that pregnancy does not represent a state of immunodepression, but conversely a state of immuno-activation [1]. Maternal lymphocytes are alerted by the presence of non-self components, but lymphocytes do not reach the stage of full activation and thus the immunological aggression of the foetus is prevented. It is worth nothing that this increase in 4F2-positive cells appears during the second and third trimesters of pregnancy when maternal exposure to the foreign antigen is maximal. Similarly, the 4F2-positive cells present in the neonate may reflect the tolerance of the foetal immune system to maternal antigenicity. An alternative explanation of this finding is that 4F2-positive cells are in fact immature foetal lymphocytes which are present in both neonatal and maternal circulations. It is known that foetal lymphocytes are found in the maternal circulation in a high percentage of cases [19, 20], a fact which has led to practical applications in tissue typing studies. Thus, 4F2-positive foetal lymphocytes enter the maternal circulation during the latter part of pregnancy and are likely to be important factors in the complex materno-foetal immunological symbiosis.

In diabetic pregnancy, in addition to the increase in the 4F2 antigen on mononuclear cells, there is an increase in MHC Class II-positive lymphocytes in Type 1 pregnant diabetic patients and in gestational diabetic women.

While the increase in Class II-positive T cells in a number of Type 1 diabetic women is likely to reflect the immune abnormalities pre-existent to pregnancy, the increase in Class II-positive T cells in gestational diabetic patients is unexpected. Attention has been recently focused on this form of diabetes, in which an increased presence of islet cell antibodies has been already reported [4, 21]. The finding of class II-positive cells in some gestational diabetic patients reinforces the concept of the immunopathological similarities of these cases to Type 1 diabetes. Indeed, gestational diabetes is a valuable model which allows the selection of some individuals prone to develop Type 1 diabetes in the pre-clinical period.

In diabetic pregnancy the cellular immune factors are simultaneously influenced, as expected, by phenomena related to diabetes and to pregnancy. It remains to be shown whether the superimposition of diabetes on normal pregnancy represents the "final straw" in immunological terms with consequent influences on the development of the foetus [17]. Indeed, the increase in Class II-positive cells in the blood of infants of diabetic mothers is intriguing. Fully mature, activated and potentially dangerous T lymphocytes are known to be present in the early stages of Type 1 diabetes. The finding of these cells in the neonatal circulation raises questions concerning their presence and potential role. Since these cells are not present in the circulation of normal neonates, it is possible to infer that activated T lymphocytes are passively transferred from the maternal to the neonatal circulation. Their possible role is less clear. These cells may simply either be an epiphenomenon of maternal disorders or have a physiopathological influence on foetal and neonatal development. It is worth noting that despite the possible presence of islet cell antibodies, insulin antibodies, insulin-anti-insulin complexes and Class II-positive T cells in their circulation, infants of diabetic mothers, including those in our studies, are not reported to develop clinical diabetes early in life. The possibility of sub-clinical metabolic or tissue modifications predisposing a long-term islet B-cell failure cannot be disregarded.

Acknowledgements. The research project was supported by a grant from the "Ministero di Pubblica Istruzione", number 2.12.1.40. The authors express their thanks to Dr. L. Byatt for editing the paper and to Ms. M. Barbieri for her excellent secretarial assistance.

References

1. Gleicher N, Siegel I (1983) Immunology of pregnancy. Wiener Klinische Wochenschrift 95, 11: 377–382
2. Shulman S, Dondero F, Nicotra M (eds) (1982) Immunological factors in human reproduction. Serono Symposia, vol 45. Academic Press, New York
3. Andreani D, Di Mario U, Federlin KF, Heding LG (1984) Immunology in diabetes. Kimpton Medical Publications, London Edinburgh
4. Andreani D, Di Mario U, Zuccarini O, Franco M, Sensi M, Iavicoli M, Pozzilli P (1984) Lymphocyte subsets and immune complexes in long standing diabetic patients: relation with the presence of microangiopathy. Immunol Lett 8: 17–21
5. Ginsberg-Fellner F, Mark EM, Nechemias C, Hausknecht RU, Rubinstein P, Dobersen MJ, Notkins AR (1980) Islet cell antibodies in gestational diabetics. Lancet 2: 362–363
6. Sensi M, Pozzilli P, Di Mario U (1984) A new method to facilitate the reading of up to 60 monoclonal antibody-fluorescence stained cells using medicell HLA-DR plates. J Clin Lab Immunol 13: 101–102
7. Eisenbarth GS, Haynes BF, Schorer JA, Fauci AS (1980) Production of monoclonal antibodies reacting with peripheral blood mononuclear cell surface differentiation antigens. J Immunol 124: 1237–1241
8. Lampson LA, Levy R (1980) Two populations of Ia like molecules on a human B cell line. J Immunol 125: 293–299
9. Guy K, Van Heyningen V, Cohen BB, Deane DL, Steel CL (1982) Differential expression and serologically distinct subpopulations of human Ia antigens detected with monoclonal antibodies to Ia alpha and beta chains. Eur J Immunol 12: 942–948
10. Di Mario U, Guy K (1984) Evaluation of class II antigen-positive T cells: a simple double staining method using monoclonal antibodies. J Clin Lab Immunol 13: 203–204
11. Tingle AJ, Lim G, Wright VJ, Dimmick JE, Hunt JA (1979) Transplacental passage of islet cell antibody in infants of diabetic mothers. Pediat Res 13: 1323–26
12. Steel JM, Irvine WJ, Clarke BF (1980) The significance of pancreatic islet cell antibody and abnormal glucose tolerance during pregnancy. J Clin Lab Immunol 4: 83–5
13. Mylvaganam R, Stowers JM, Steel JM, Wallace J, MacHendry JC, Wright AD (1983) Insulin immunogenicity in pregnancy: maternal and fetal studies. Diabetologia 24: 19–25
14. Fallucca F, Di Mario U, Gargiulo P, Iavicoli M, Galfo C, Contreas G, Pachì A, Andreani D (1985) Humoral immunity in diabetic pregnancy: interrelationships with maternal/neonatal complications and maternal metabolic control. Diab Metab 11: 386–395
15. Theophilopoulos AN, Gleicher N, Pereira AB, Dixon FJ (1981) The biology of immune complexes and their possible role in pregnancy. Am J Reprod Immunol 1: 92–105
16. Vazquez-Escobosa C, Perez-Medina R, Gomez-Estrada H (1983) Circulating immune complexes in hypertensive disease of pregnancy. Obst Gynecol 62: 45–48
17. Faulk WP, Galbraith RM, Keane M (1980) Immunological consideration of the feto-placental unit in maternal diabetes. In: Irvine WJ (ed) Immunology in diabetes. Teviot, Edinburgh, pp 309–317
18. Di Mario U, Fallucca F, Gargiulo P, Tiberti C, Scardellato A, Arduini P, Pachì A, Andreani D (1984) Insulin-anti-insulin complexes in diabetic women and their neonates. Diabetologia 27: 83–86
19. Siegel I, Gleicher N (1980) The development of the fetal immune system. Mount Sinai J Med 47: 474–480
20. Gill TJ (1977) Chimerism in humans. Transplant Proc 9: 1423–1431
21. Iavicoli M, Fallucca F, Di Mario U, Tinelli F, Trovati A, Colletti A, Montenero P, Andreani D (1980) Islet cell antibodies in the offspring of two diabetic parents. In: Pinchera A, Doniach D, Fenzi GF, Baschieri L (eds) Autoimmune aspects of endocrine disorders. Academic Press, London, pp 311–312

Received: 23 April 1986
and in revised form: 30 December 1986

Dr. U. Di Mario
Clinica Medica 2 (Endocrinologia 1)
Università/Policlinico Umberto I
I-00161 Rome
Italy