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Antibody dependent cellular cytotoxicity (ADCC)-reaction and an in vitro steroid sensitivity test of peripheral lymphocytes in children with malignant haematological and autoimmune diseases

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ADCC reaction (antibody dependent cellular cytotoxicity), ADCC capacity and ADCC steroid sensitivity examinations were performed in 20 children with tumorous or haematological diseases, 10 children with autoimmune diseases, and appropriate controls, in order to establish the killer function and steroid sensitivity. In the above diseases a study was also made of the correlation of the individual reactions with the duration of steroid therapy.

The two patient groups did not exhibit a significant difference from the controls as concerns the ADCC reaction and ADCC capacity.

In the group of tumorous or malignant haematological diseases the steroid sensitivity behaved in a different way, with sensitivity in 45%, moderate sensitivity in 20%, and steroid resistance in 35% of the patients. Steroid inhibition of the ADCC reaction was significantly decreased in the group of autoimmune patients.

There was no correlation between ADCC reaction and ADCC steroid sensitivity or resistance in either group, and thus the ADCC steroid resistance or sensitivity and lymphocyte killer function proved to be independent. No correlation was found between the steroid sensitivity or resistance and the duration of steroid treatment.

Natural killer (NK) and killer (K) cells are known to play important roles in non-specific antitumorous immunological defence [1, 5, 17]. Explorations of the relationship between K and ADCC effectors [1, 10] have led to the suggestion that NK and ADCC effectors are probably the same. It has been shown that these cells may bear T-cell and/or myelomonocytic markers and that they can be characterized morphologically as large granular lymphocytes which are mostly present in the low-density Percoll fractions [21]. Interferon treatment increases their activity, while mediators such as prostaglandins, steroids and cytophilic immunoglobulins inhibit the activity [12].

Previous publications reported on diminished NK activity or ADCC activity in patients with malignant breast, liver, pancreas or colon tumours, and in melanoma malignum [6, 7, 17, 18, 19].

We have investigated the killer activity of lymphocytes and steroid inhibition of the ADCC reaction in children suffering from malignant diseases. The correlation between the in vitro effect of steroid on the ADCC reaction and the duration of steroid therapy was also analysed.

Patient Material

Steroid sensitivity was measured in the traditional ADCC reaction (number of effector cells in excess) [22] and in the ADCC-capacity (ADCC-C) test (excess of target cells) in 20 children suffering from haematological diseases or tumour (Group I, Table II), 10 children with autoimmune diseases (Group II, Table III) and controls.

Methods

Cytotoxic capacity of lymphocytes (ADCC-C). The essence of the method is as follows. In the event of a high number of target cells the cytotoxic activity of lymphocytes increases, and thus maximum cytotoxic activity (capacity) of the cell population may be measured by titration of the target cells.

A three times washed suspension of freshly taken Rh (D, C)-positive "O" red blood cells was used as *target cells.* ^{51}Cr labelling was performed in the presence of Na-citrate, by the addition of 100 μCi $Na_2{}^{51}CrO_4$ (Amersham; spec. act. 7—8 GBq/mg Cr) for 60 minutes at 37°C. After labelling, the cells were washed 3 times, and the cell count was adjusted to 10^7/ml. The *effector lymphocytes* were isolated on a Ficoll-Uromiro gradient after treatment of the whole blood with colloidal iron powder (GAF, USA).

50 μl quantities of serial 1 : 2 dilutions of the target cells were added per culture in microtiter plates (Greiner), together with 50 μl of a 2×10^6/ml suspension of effector lymphocytes. Thus, while the effector cell count remained constant (10^5 lymphocytes), the target cell count decreased due to the 1 : 2 dilution.

For one test, 50 μl of a 1 : 20 dilution of incomplete anti-D antibody was added, and the volume was made up to 200 μl with 50 μl RPMI medium (Serva) containing 10% calf serum (Herman, Budapest). The cultures were incubated in a CO_2 thermostat for 18 hours at 37°C, and the activity of 100 μl supernatant was measured. The steroid (methylprednisolone) was added together with the supplementary nutrient medium, in a final concentration of 5—10 μg/ml.

For the determination of total activity, measurements were made of the radioactivity of 25 μl labelled red blood cells. The spontaneous activity was given by the count rates for steroid-containing cultures without anti-D antibody.

The means of the count rates for 3—4 parallel samples were used for evaluation. No. of target cells destroyed:

$$\frac{\text{No. of target cells added} \times \text{cytotoxicity}}{100}$$

Steroid sensitivity studies were carried out by means of target cells excess cytotoxic capacity investigations, together with the effector excess traditional *cytotoxic reaction,* ADCC. In the latter reaction all conditions were similar (culture medium, target cell, time of incubation) to those described in the ADCC-C test, the only difference was the effector/target ratio being 10 : 1.

Steroid sensitivity or resistance was measured by the percent inhibition of the ADCC reaction.

ADCC inhibition $<30\%$, resistant to steroid

ADCC inhibition 30—50%, moderate sensitivity to steroid

ADCC inhibition $>50\%$, steroid sensitivity

Results

Table I presents the results of ADCC-reaction and ADCC-capacity examinations in children with malignant tumour or haematological disease or with autoimmune diseases. These two groups of patients did not exhibit significant differences from the controls in the ADCC reaction and the ADCC capacity. In the group with tumour and malignant haemato-

logical disease, the ADCC reaction was markedly decreased, to below 35%, in 4 of the initial 10 cases. The ADCC reaction was above 30% in all children in the autoimmune group, while it proved low (4% and 13%) in two subsequent cases not shown in Table I; a pronounced steroid sensitivity was confirmed in these cases.

Table II details the percentage of the steroid inhibition of ADCC in the two groups, as an indication of the steroid sensitivity or resistance. The clinical diagnosis and the treatment are also listed, with emphasis on whether the children had participated in steroid treatment. Varying results were obtained concerning the in vitro sensitivity in the malignant tumorous and haematological patients: 9/20 displayed sensitivity, 4/20 moderate sensitivity, 7/20 steroid resistance, and thus the group average did not differ from that for the control group. Seven of the 13 steroid-sensitive or moderately sensitive patients had not received steroid before the examination. In the group of autoimmune diseases, a significantly decreased ADCC steroid inhibition was found after prednisolone treatment, as compared to the controls (Table III).

Investigation of a possible connection between ADCC capacity and ADCC steroid sensitivity or resistance of the patients revealed that there was no correlation between the percentage of steroid inhibition of ADCC and ADCC capacity in either the malignant tumorous group or the autoimmune group (Table IV), i.e. ADCC steroid resistance or sensitivity and killer capacity proved to be independent. The Mann—Whitney test did not demonstrate a significant connection between ADCC reaction and steroid sensitivity or resistance in the tumorous group ($r = 0.46$; $p > 0.1$) or in the autoimmune group ($r = -0.21$; $p > 0.1$). Nor was a significant correlation found between ADCC steroid sensitivity or resistance and the duration of steroid treatment for either group (tumorous group: $r = -0.08$; $p > 0.1$; autoimmune group: $r = -0.15$; $p > 0.1$).

TABLE I

Results of ADCC-reaction and ADCC-capacity

Group of patients suffering from malignant tumors and haematological diseases		Group of autoimmune patients		Controls	
ADCC-reaction per cent	ADCC-capacity	ADCC-reaction, per cent	ADCC-capacity	ADCC-reaction, per cent	ADCC-capacity
(n = 10)	(n = 15)	(n = 9)	(n = 10)	(n = 21)	(n = 16)
T = ± 40,2	29783.3	52	34520	41.46	23593.7
S.D. i 23.9	26393.12	15.06	35081	14.06	20396
p > 0.05	(Mann—Whitney's test				

TABLE II

Result of ADCC-steroid inhibition test

Sign	Age year	Sex	ADCC inhibition per cent	Sensitive (2 points)	Moderately sensitive (1 point)	Resistant (0 point)	Clinical diagnosis	Therapy (duration in weeks)
Children's group suffering from malignant haematological diseases or tumors								
U.T.	7	♂	69,5	+			Non-Hodgkin lymphoma (NHL)	—
N.B.	5	♀	57	+			Acute lymphoid leukaemia (ALL)	Prednisolone (3 wk) MTX, Kidrolase, Cytosar, Cyclophosphanide
N.E.	2	♀	10			+	ALL	Prednisolone (8 wk)
B.I.	2.5	♀	29			+	Neuroblastoma	Prednisolone (6 wk) VCR, Cyclophosphamide DTIC
O.I.	5	♀	0			+	NHL	Kidrolase
U.A.	8	♂	17			+	Reticuloendothelioma	VAC
N.B.	5	♂	40		+		ALL	Prednisolone (7 wk) MTX
L.Sz.	4	♀	51	+			Wilms tumour	—
B.A.	4	♀	20.5			+	Neuroblastoma	Prednisolone (5 wk) VAC
Z.K.	8	♀	41.5		+		Neuroblastoma	—
K.L.	10	♂	55	+			ALL	Prednisolone (2 wk)
D.L.	8	♂	42		+		Eosinophil granuloma	Prednisolone (2 wk)
M.P.	3	♂	74	+			ALL	—
M.I.	5	♂	85	+			ALL	—
H.L.	10	♂	15			+	ALL	Prednisolone (12 wk) VCR Cytosar, Kidrolase, MTX, VP 16
P.I.	9	♂	79	+			ALL	Prednisolone (16 wk) MTX, Rubidomycin, Kidrolase
Ö.T.	6 m.	♂	40		+		Hepatoblastoma	—
F.T.	2	♀	80	+			Ependymoma	—
B.H.	4	♀	70	+			ALL	Prednisolone (4 wk) MTX
A.K.	9	♂	15			+	Rhabdomyosarcoma	—
(n = 20	$\bar{X}$ =		44.52	$p > 0.05$ (Mann—Whitney's test)				
	S.D. ±		26.24					
	X =		53.08					
Controls								
(n = 28)	S.D. ±		13.16					

TABLE III

Result of ADCC-steroid inhibition test

Sign	Age year	Sex	ADCC inhibition per cent	Sensitive (2 points)	Moderately sensitive (1 point)	Resistant (0 point)	Clinical diagnosis	Therapy (weeks)
Group of children suffering from autoimmune diseases								
H.L.	15	♂	56.5	+			Aplastic anaemia	Prednisolone (5 weeks) Anapolon
F.J.	9	♂	20			+	Hamman—Rich	Prednisolone (6.5 weeks)
E.É.	6	♀	0			+	Rheumatoid arthritis	Prednisolone (7.5 weeks)
F.E.	13	♀	0			+	Ileitis (Crohn)	Prednisolone (8 weeks) Salazopyrine
Sz.G.	4	♀	35		+		Dermatomyositis	Prednisolone (9.5 weeks)
B.P.	12	♀	61.5	+			ITP	Prednisolone (2 weeks)
K.Cs.	9	♂	48.5	+			ITP	Prednisolone (0.5 week)
V.A.	7	♀	9.5			+	Rheum. arthritis	Prednisolone (4.5 weeks)
R.R.	14	♂	36		+		Rheum. arthritis	—
K.A.	7	♀	4			+	Rheum. arthritis	—
	$\bar{X}$ =		27.1					
	S.D. ±		23.6 (n = 10)	$p < 0.01$ (Mann—Whitney's test)				
Controls								
	$\bar{X}$ =		53.08					
	S.D. ±		13.16 (n = 28)					

TABLE IV

Mathematical evaluation of the ADCC-steroid inhibition test and ADCC-capacity

Group of malignant tumours or haematological diseases	
Correlation between percentual ADCC-steroid inhibition and ADCC-capacity	$r = -0.33$ $p > 0.05$
Group of autoimmune diseases	
Correlation between percentual ADCC-steroid inhibition and ADCC-capacity	$r = -0.15$ $p > 0.05$

DISCUSSION

Children with malignant tumour or autoimmune disease did not exhibit a difference from the controls as concerns the ADCC reaction or the ADCC-C value. The lymphocyte population participating in the ADCC reaction displayed an appreciable overlap with the lymphocytes responsible for the NK reaction. The literature on NK activity in autoim-

mune diseases shows that NK activity is reduced in SLE and rheumatoid arthritis [8, 9, 15, 20], while authors [12] described an enhancement of NK activity. We did not find any killer activity change in either the ADCC reaction or the ADCC-capacity.

Numerous data point to the decreased NK and ADCC activity in adult patients with tumour [4, 6, 7, 17, 18, 19]. In our children with malignant disease or leukosis we found no significant differences from the normal. There was no correlation between killer capacity (ADCC—C) and steroid inhibition of the ADCC reaction in cases of malignant haematological disease or tumour.

The steroid inhibition of ADCC or resistance differed from that of the controls in most cases of malignant tumour, and particularly in steroid-treated cases. ADCC steroid inhibition was significantly decreased in children suffering from autoimmune diseases. As an explanation, the idea emerged that prednisolone treatment during several months caused the in vivo prednisolone-sensitive lymphocyte subpopulation to decrease or their steroid receptors to be blocked; accordingly, further inhibition could not be induced in response to prednisolone in an in vitro test. Another possibility is that steroid resistance may have existed even before prednisolone treatment. Rank-correlation analysis did not reveal a significant correlation between the duration of steroid treatment and the steroid sensitivity or resistance, thus there was no indication of a steroid resistance existing initially in some of the cases. The question arises of whether it is worthwhile to apply prednisolone in all steroid-resistant cases.

Since mainly the IgG Fc receptor-bearing lymphocytes take part in the ADCC reaction, it is conceivable that the interindividual differences in steroid sensitivity may be correlated with a shift in the proportion of the T_G lymphocyte subpopulation.

In patients with cancer [17] and malignant diseases [23], there is much evidence pointing to cellular immunity, that the T cell growth factor (TCGF) is involved in these abnormalities. Grabtree et al [4] presented considerable evidence that glucocorticoids inhibit T cell proliferation by blocking the production of TCGF. Parillo and Fauci [16] found that corticosteroids suppressed NK activity in the human. Similarly, NK activity is known to be reduced in kidney allograft recipients treated with prednisolone [11].

A cytotoxic T cell line could destroy tumour cells only if the TCGF was present in sufficient amounts in the test system[3]. A TCGF level reduced by corticosteroids might be a reason for the lack of effect of T cells in cancer patients. The decreased TCGF production is closely related to tumour progress in patients with cancer [14]. Steroid administration should be considered with regard to the individual patient in the event of an initially decreased T cell function, in order to avoid a further reduction of T cell functions.

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