Acta Paediatrica Hungarica, 26 (3), pp. 247—253 (1985)

An in vitro steroid sensitivity test: antibody-dependent cellular cytotoxicity (ADCC) reaction of peripheral lymphocytes in children with nephrotic syndrome

Ildikó Petri, Aranka László, T Bodrogi

Blood Transfusion Centre, and Department of Paediatrics, University Medical School, Szeged, Hungary

A steroid effect is described that can be measured in vitro; this was determined by means of the antibody-dependent cellular cytotoxicity (ADCC) method. Examinations on 15 children with nephrotic syndrome revealed a significant correlation between the steroid sensitivity measured in vitro and the clinical sensitivity to prednisolone.

The in vitro measurement of steroid sensitivity yielded fast and reliable information on the effectivity of prednisolone treatment.

In the antibody-dependent cellular cytotoxicity (ADCC) test, an IgG antibody on a target cell serves as the recognition link for the Fc-IgG receptor carrying effector cell. Several cell types of different linkages have been shown to act as effectors: neutrophils, eosinophils, monocytes and lymphocytes. Whereas all the ADCC effectors, which have been named K cells, are Fc-IgG (+), only about half of the Fc-IgG (+) lymphocytes can function in ADCC [22] and these are probably high avidity EA rosette formers. The first evidence for a Null cell-mediated ADCC activity came from Wisloff and Froland [23] who showed that lysis was independent of B and T cells. Using various other methods for purification of the Null subset, Horwitz et al [7] isolated L lymphocytes which did not form rosettes with sheep erythrocytes and lacked membrane stable Ig but possessed an Fc receptor and had the capacity to kill antibody-coated target cells. Data from Takasugi's group have been interpreted to the effect that natural killer (NK) cells were indeed K cells, but "armed" with natural antibodies in vivo [9].

Many of the investigators exploring the relationship between K and ADCC effectors [2, 10] suggested that NK and ADCC effectors were probably the same. This was supported by recent investigations with monoclonal antibodies: anti-human Leu 7 (HNK-1) reacted with both NK and K cells [1]. It has been shown that these cells may bear T-cells and/or myelomonocytic markers and that they can be characterized morphologically as large granular lymphocytes (LGL) which are mostly present in the low-density Percoll fractions [19]. Inter-

feron treatment increased their actvity, while other mediators such as prostaglandins, steroids and cytophilic immunoglobulins inhibited their activity [11].

The ADCC resistance or sensitivity was studied earlier in renal transplant recipients with in vitro methylprednisolone, and it was found that the allograft survival time is correlated with the result of the above test, i.e. it reflected the in vivo steroidresistant or sensitive state [20, 15, 8, 4, 5].

We have performed an investigation of the ADCC reaction to assess the steroid sensitivity of children with nephrotic syndrome, a comparison being made with the clinical effect of steroid therapy.

Patients and Methods

ADCC activity was determined on 16 occasions in 15 children with nephrotic syndrome who were patients of the Nephrology Unit of the Department of Paediatrics, University Medical School, Szeged. The method detailed below was used. 13 healthy young blood donors served as controls.

Separation of effector cells

The effector lymphocytes were isolated on a Ficoll Uromiro gradient [3] after treatment of the whole blood with colloidal iron powder (GAF, USA), from 10 ml venous blood taken with heparin. The lymphocyte suspension was adjusted to 5×10^6/ml in RPMI 1640 culture medium containing 10% FCS.

Performance of ADCC reaction

Fresh, human „O" Rh (D) positive red blood cells were used as target cells. Human anti-D serum was adsorbed onto the cells [21] labelled with $^{51}Cr/Na_2\ ^{51}CrO_4$; 7—8 GBq/mg Cr; Amersham). The effector: target cell ratio was then adjusted to 20 : 1 or 10 : 1. Methylprednisolone was then added to the culture medium in a final concentration of 5—10 μg/ml, the cells were incubated at 37°C in a 5% CO_2 thermostat for 18 hours. The cytotoxicity was calculated from the activity of the supernatant by the formula

$$\text{cytotoxicity \%} = \frac{\text{test supernatant cpm} - \text{spontaneous cpm}}{\text{incorporated total activity cpm}} \times 100$$

The steroid sensitivity tests were carried out not only with the traditional effector excess cytotoxic reaction, but also with the target cell excess cytotoxic capacity test [6, 14].

Steroid sensitivity or resistance was given as a percentage of the inhibition of the ADCC reaction due to the steroid. The ranges were as follows.

<30%	ADDC inhibition: steroid resistant
30—50%	ADDC inhibition: moderately steroid-resistant
>50%	ADCC inhibition: steroid-sensitive

Results

The results of the ADCC inhibition test are given in Table I. In vitro steroid sensitivity was confirmed in every patient with nephrotic syndrome (NS) with a clinical prednisolone sensitivity of ++++. Special mention should be made of patient H. H., in whom the steroid sensitivity found clinically was +++, which

TABLE I

Results of ADCC test in children with nephrotic syndrome

Initial	Age in years	Sex	ADCC inhibition per cent	Sensitive	Moderately sensitive	Resistant	Renal biopsy diagnosis	Maximum proteinuria g/day	Prednisolone sensitivity	Treatment
B. M.	5	♀	45—64	+			MSGN	9.1	++++	Pr. Pr + Chl
K. Zs.	6	♂	33—41		+		(NS)	4.3	++	Pr
Sz. R.	10	♂	0			+	MSGN/MPGN	6.3	0	Pr, Pr + Chl
N. G.	9	♂	50—67	+			MSGN (Sch.HH)	3.26	++	Pr, Pr + Chl
B. Zs.	10	♀	41—50		+		MSGN	23.0	0	Pr, Pr + L. Pr + Chl + hep.
Gy. Z.	8	♂	50	+			MCNS	2.4	++++	Pr, Pr + Chl
Sz. É.	3	♀	50	+			MCNS/FSGN	2.5	++	Pr, Pr + Chl
B. Zs.	10	♀	33			+	MSGN	22.0	0	Pr + L + hep dipyridamide
U. P.	5	♂	36			+	MSGN	5.3	0	Pr + L + indomethacin
K. I.	7	♂	60—74	+			(NS)	3.0	++++	Pr
F. R.	6	♀	48—57	+			MCNS	4.0	+	Pr, Chl
N. E.	11	♀	35—44		+		MSGN (Sch.H)	4.5	0	Pr, Pr + Chl
H. H.	6	♀	15—30			+	MCNS	8.7	+++−+	Pr, Pr + Chl
P. E.	5	♀	53	+			MCNS	1.0	++++	Pr
V. A.	4	♂	34—56		+		(NS) (Sch.H)	3.2	++	Pr
F. P.	9	♂	9—27			+	(NS)	1.5	0	Pr

NS = nephrotic syndrome
Sch.H = Schönlein-Henoch
MCGN = minimal change glomerulonephritis
MSGN = mesangioproliferative glomerulonephritis
MPGN = membranoproliferative glomerulonephritis
FSGN = focal sclerotic glomerulonephritis
Pr = prednisolone
Chl = chlorambucil

TABLE II

Mathematical evaluation of ADCC inhibition test

Control		ADCC inhibition, per cent	Prednisolone sensitivity clinically (in vivo) 0 = 0 points + = 1 point ++++ = 4 points	Prednisolone sensitivity (in vitro) resistant = 0 points moderately sensitive = 1 point sensitive = 2 points
(n = 13)		(n = 16)	(n = 16)	(n = 16)
$\bar{X}$ =	61.07	41.37	1.68	1.12
S.D. ±	9.34	16.9	1.6	0.88
difference	$p < 0.001$	corr. coeff. = 0.71		corr. coeff. = 0.76
		$p < 0.01$		$p < 0.001$

had decreased to + by the time of the in vitro examination.

The change in percentage ADCC inhibition was studied in correlation with the morphological change in the kidney and with the clinical picture, i.e. with the sensitivity displayed to prednisolone treatment. The correlation coefficients were calculated by taking into consideration the number of NS attacks and the duration and effectivity of steroid treatment.

The ADCC inhibition test demonstrated that 7 nephrotic syndrome patients were steroid-sensitive, 4 cases were moderately sensitive, and 5 cases were steroid-resistant.

A significant correlation was found between the result of the in vitro steroid sensitivity test and clinical prednisolone sensitivity in the steroid-sensitive group, in the moderately sensitive group, and in the steroid-resistant group (Table II).

The in vitro steroid resistance exhibited a good correlation in the NS cases with a renal biopsy finding of MPGN or MSGN, involving a serious prognosis. Only in one MCNS patient was there a contradiction; this was H. H., where in vitro steroid resistance was observed in spite of the slight biopsy finding. From among the NS patients demonstrated to be steroid-sensitive in vitro, 2 patients with a renal biopsy finding of MSGN gave a partially contradictory correlation for the clinical picture and the sensitivity; for other 5 steroid-sensitive NS patients the MCNS biopsy finding correlated well with the result of the in vitro test.

2 of the 4 moderately steroid-sensitive NS cases belonged in the serious MSGN group.

CASE REPORTS

Brief accounts of our NS cases are provided below for the purpose of evaluation of the response given to prednisolone.

B. M. (born 18.02.1979), female. Diagnosis: MSGN. Maximum proteinuria without prednisolone: 9.1 g/day. All 5 recurrences responded well to prednisolone, but because of the frequent relapses, alternating prednisolone and chlorambucil treatment was administered for 6 weeks during the last recurrence. Proteinuria-free since combined treatment.

K. Zs. (born 18.01.1978), male. Diagnosis: NS. Bilateral renal hypoplasia, bilateral megaureter. Maximum proteinuria: 4.3 g/day. Renal biopsy: not informative. The proteinuria decreased substantially in response to prednisolone, though it still persists at 0.25—0.5 g/day.

Sz. R. (born 29.09.1973), male. Diagnosis: MSGN from first renal biopsy in April, 1980; MSGN/MPGN, from second renal biopsy in November, 1982. An exact classification was not possible, but at any event the state corresponded to immune complex nephritis. Maximum proteinuria: 6.3 g/day. Prednisolone alone was ineffective. Prednisolone supplemented with chlorambucil led to moderation of the proteinuria. After a biopsy in 1982, prednisolone administration was stopped. The proteinuria is moderate, at present it is 1.8—2.5 g/day.

N. G. (born 10.04.1975), male. Diagnosis: MSGN (Schönlein-Henoch). Maximum proteinuria: 3.26 g/day. Prednisolone alone only moderated the proteinuria; prednisolone combined with chlorambucil eliminated it.

B. Zs. (born 13.02.1974), female. Diagnosis: MSGN. Maximum proteinuria: 23 g/day. The proteinuria has proved resistant to therapeutic efforts. Treatments: Prednisolone; prednisolone + chlorambucil; prednisolone + chlorambucil + heparin; prednisolone + chlorambucil + heparin + dipyridamole; plasmapheresis on 4 occasions.

Gy. Z. (born 29.12.1972), male. Diagnosis: MCNS. Maximum proteinuria: 2.4 g/day. Responded well to prednisolone in all cases but, due to recurrences, chlorambucil too, was administered for 6 weeks.

Sz. É. (born 08.12.1982), female. Diagnosis: MCNS/FSGN. Maximum proteinuria: 2.5 g/day. During prednisolone treatment, the proteinuria was intermittent; accordingly, chlorambucil administration too was begun. Proteinuria currently, 1.5 g/day.

U. P. (born 19.05.1979), male. Diagnosis: MSGN. Maximum proteinuria: 5.3 g/day. Proved steroid-resistant clinically. The proteinuria responded only to prednisolone + chlorambucil + indomethacin treatment. Proteinuria currently, 0.5—1.0 g/day.

K. I. (7 years old), male. NS began 3 years ago, there have been 4 recurrences. Maximum proteinuria: 3.0 g/day. Renal biopsy was not performed. A rapid and good response to steroid in all cases.

F. R. (6 years old), female. Diagnosis: nephroso-nephritis, MCNS. Albuminuria initially accompanied by massive haematuria. Maximum proteinuria: 4.0 g/day. Had steroid treatment for 3 months, during which marked hypertension and obesity developed, and proteinuria decreased to 1 g/day. Subsequently only chlorambucil was administered. Renal biopsy confirmed MCNS.

N. E. (11 years old), female. Diagnosis: MSGN (Schönlein-Henoch). Maximum proteinuria: 4.5 g/day. The proteinuria was unchanged in response to prednisolone treatment, but decreased somewhat when prednisolone was supplemented with chlorambucil.

H. H. (born 04.04.1978), female. Diagnosis: MCNS. Maximum proteinuria: 8.7 g/day. Reacted well to steroid in all cases, though the fourth recurrence took place during inadequate alternating steroid treatment. Renal biopsy was performed during the fifth recurrence; finding: MCNS. Therapy: prednisolone + chlorambucil.

P. E. (born 25.10.1979), female. Diagnosis: MCNS. Maximum proteinuria: 1 g/day. Only prednisolone is administered.

V. A. (born 04.03.1980), male. Symptoms of NS began in March, 1984, in connection with Schönlein-Henoch nephropathy. Maximum proteinuria: 3.2 g/day. 60 mg/m² steroid treatment started on 27.04.1984. The proteinuria improved to 1.4 g/day, but steroid treatment did not eliminate it.

F. P. (born 29.01.1975), male. Admitted with suspicion of focal nephritis. Maximum proteinuria: 1.5 g/day. Participates in 60 mg/m² steroid therapy since 16.05.1984, but the proteinuria is unchanged.

Discussion

Our examinations revealed a significant correlation between the clinical effect of prednisolone treatment, i.e. the prednisolone sensitivity, and the degree of steroid sensitivity or resistance based on the percentage ADCC inhibition in children with NS. Mathematical evaluation showed that the inhibition values obtained with steroid in the blood donors used as controls were almost the same at different target: effector cell ratios both in the ADCC reaction and in the ADCC capacity test, and thus the percentage inhibition could be expressed as a concrete number. At the same time, the percentage inhibition values obtained at different target: effector cell ratios in the case of NS patients were partly different; accordingly, the percentage inhibitions obtained with steroid were given as ranges, which results in a better reflection of the general steroid sensitivity.

The in vitro value of steroid sensitivity or resistance was fairly constant in time (when control examinations were carried out in intervals of 2—3 months) and was well reproducible [14]. For this reason, sensitivity tests were performed only once in the present series of examinations. The only exception was B. Zs., a MSGN patient, in whom the test was repeated after an interval of 2 months.

As concerns the explanation of the steroid effect, one of our conceptions is that the in vitro and in vivo actions of the steroid may be connected with the number of steroid receptors on the surface of lymphocytes, or with the blocking of the receptors.

Szekeres et al (17) have demonstrated that the progesterone binding ability of lymphocytes is of informative value from the aspect of the outcome of pregnancy.

Investigations into the effects of monoclonal antibodies on in vitro immune functions showed that interleukin-2 (IL-2) played important roles in the manifestation of the suppressor and helper activities exerted by T lymphocytes [12]. It has also been demonstrated that the steroids exert their suppressive action during the interaction of interleukins and T lymphocytes [13]. At the International Transplantation Congress in Brighton, Rosenbert et al [15] described a selective effect of methylprednisolone, which was detected in the interaction of IL-2 and T lymphocytes. As a key role is attributed to the HLA-DR antigens in the effect of interleukins, this would explain the manifestation of both steroid sensitivity and steroid resistance in normal individuals. In the future, therefore, we plan to compare the HLA-DR types of healthy subjects with the steroid effect exhibited in vitro.

References

1. Abo T, Blach CM: A differentiation antigen of human NK and K cells identified by a monoclonal antibody (HNK-1). J Immunol 127: 1024, 1981
2. Benczur M, Győrffy G, Garam T, Varga M, Medgyesi G, Sándor M,

Petrányi GG: Correlation between effector lymphocytes in natural and antibody-mediated cytotoxicity. Immunobiology 156: 320, 1979
3. Böyum A: Separation of leukocytes from blood and bone marrow. Scand J Clin Lab Invest 21: 1, 1968
4. Dumble LJ, MacDonald IM, Kincaid-Smith P: Human renal allograft rejection is predicted by serial determinations of antibody-dependent cellular cytotoxicity. Transplantation 29: 30, 1980
5. Dumble LJ, Macdonald IM, Kincaid-Smith P, Clunie GJ: Correlation between ADCC resistance to in vitro steroid and renal allograft failure. Transplant Proc 13/3 1569—71, 1981
6. Garam T, Bakács T, Varga M, Petrányi Gy: Emberi lymphocyták human vörösvérsejtek elleni cytotoxikus kapacitásának vizsgálata. Orv Hetil 121: 1705, 1980
7. Horwitz DA, Garret MA: Distinctive functional properties of human blood L lymphocytes: A comparison with T lymphocytes, B lymphocytes, and monocytes. J Immunol 118: 1712, 1977
8. Jeannet M, Vassali P: The role of lymphocyte-dependent antibody in kidney transplantation. Transplantation 22: 493, 1976
9. Koide Y, Takasugi M: Determination of specificity in natural cell-mediated cytotoxicity by natural antibodies. J Natl Cancer Inst 59: 1099, 1977
10. Landazuri de MO, Silva A, Alvez J, Herberman RB: Evidence that natural cytotoxicity and antibody-dependent cellular cytotoxicity are mediated in humans by the same effector cell populations. J Immunol 123: 252, 1979
11. Matheson DS, Green B, Hoar DI: The influence of methotrexate and thymidine on the human natural killer cell function in vitro. J Immunol 131: 1619, 1983
12. Palacios R: Mechanisms of T cell activation: Role and functional relationship of HLA-DR antigens and interleukins. Immunol Rev 63: 73, 1982
13. Palacios R, Kezth G, Heyningen van V: Monoclonal antibodies against HLA-DR antigens acting on stimulator cells prevent OKT 8+ T lymphocytes from acquiring sensitivity to II-2 and expressing suppressor function Eur J Immunol 13: 64, 1983
14. Petri I, Csajbók E, Kiss É, Kaiser G, Szenohradszky P: Use of ADCC reaction to study steroid sensitivity in chronic uraemic and renal transplant patients. Haematologia (Budapest) (in press)
15. Rosenber JC, Kaplan MP: Suppression of interleukin-2 synthesis by methylprednisolone and its relationship to steroid suppression of lymphocyte proliferation. Abstr. IX. International Congress of the Transplantation Society, F 17 3, Brighton 1982
16. Strom TB, Tilney NL, Carpenter CB: Identity and cytotoxic capacity of cells infiltrating renal allograft. N Engl J Med 292: 1257, 1975
17. Szekeres-Bartho J, Csernus V, Hadnagy J, Pácsa AS: Immunosuppressive effect of progesterone depends on the progesterone binding capacity of the lymphocytes. J Reprod Immunol 5: 81, 1983
18. Thomas J, Thomas F, Mendez-Picon G, Lee H: Immunological monitoring of long-surviving renal transplant recipients. Surgery 81: 125, 1977
19. Timonen T, Orlando JR, Herbermann RB: Analysis by a single cell cytotoxicity assay of natural killer (NK) cell frequencies among human large granular lymphocytes and of the effects interferon on their activity. J Immunol 128: 2514, 1982
20. Ting A, Terasaki PI: Lymphocyte-dependent antibody cross-matching for transplant patients. Lancet 1: 304, 1975
21. Urbaniek SJ: Lymphoid cell dependent (K-cell) lysis of human erythrocytes sensitized with Rhesus alloantibodies. Br J Haematol 33: 409, 1976
22. Wählin B, Perlmann H, Perlmann P: Analysis by aplaque assay of IgG or IgM-dependent cytotoxicity lymphocytes in human blood. J Exp Med 144: 1375, 1976
23. Wilsloff F, Froland SS: Antibody-dependent lymphocyte-mediated cytotoxicity in man: No requirement for lymphocytes with membrane-bound immunoglobulin. Scand J Immunol 2: 151, 1973

Received 25 August 1984

A László MD
Pf 471
6701 Szeged, Hungary