Polyamine-polyamine oxidase interaction: part of maternal protective mechanism against fetal rejection

DAVID M L MORGAN, GEORGE ILLEI

Summary and conclusions

Human retroplacental blood serum significantly ($p < 0.01$) suppresses the in-vitro uptake of ^3H -thymidine—that is, synthesis of deoxyribonucleic acid—by spontaneously growing human lymphocytes in the presence of exogenous spermine, but only in concentrations with a higher polyamine oxidase activity than that found in maternal peripheral blood serum during pregnancy.

These findings together with observations that the placenta is rich in spermine and that interaction of polyamine oxidase and substrate arrests cell proliferation suggest that such interaction might represent a localised immunoregulatory mechanism in the placental bed, which might contribute to the protection of the fetoplacental unit from possible maternal immune rejection.

Introduction

Because of paternally inherited histocompatibility antigens the conceptus may be likened to an intrauterine allograft that the mother, somehow, fails to reject. Several hypotheses have been put forward to explain this phenomenon. For example, the presence in pregnancy serum of factor(s) able to inhibit mitogen-induced lymphocyte proliferation in vitro has been reported¹⁻⁵; the inhibition occurs whether lymphocytes from pregnant or normal individuals are used,⁶ thus excluding a possible specific maternal defect of lymphocytes. Alpha-fetoprotein,⁵⁻⁷ human chorionic gonadotrophin,⁸ immune complexes,⁹ and pregnancy-specific β_1 -glycoprotein¹⁰ have all been suggested as the causative agent(s).

It has been reported that the polyamines spermine and spermidine in the presence of calf or fetal calf serum reversibly suppress mitogen-stimulated lymphocyte proliferation.¹¹⁻¹³ Calf serum possesses abundant polyamine oxidase activity¹⁴ and can convert polyamines into active inhibitors of lymphocyte proliferation. Gaugas and Curzen suggested that specific polyamine oxidases are present in pregnancy serum¹⁵ but did not directly analyse polyamine oxidase activity. Recently we have established¹⁶ the existence of a spermine-oxidising enzyme in human pregnancy serum whose activity increases as gestation progresses. Moreover, interaction of pregnancy serum diamine oxidase with substrate arrests lymphoblastic transformation.¹⁶ We have also shown¹⁷ that the polyamine oxidase activity in retroplacental serum (mainly of intervillous origin) is 20-30 times higher than that in peripheral maternal blood serum. We have therefore investigated the effects of maternal retroplacental serum with a known polyamine oxidase activity on the uptake of ^3H -thymidine (an indication of deoxyribonucleic acid synthesis) by cultured lymphoid cells.

Materials and methods

Triplicate cultures (20 μl) of the lymphoid cell lines CCRF-CEM¹⁸ and HOM-2¹⁹ (obtained from Dr M Steel, MRC Clinical and Population Cytogenetics Unit, Edinburgh) were prepared at different cell concentrations (4×10^5 , 2×10^5 , and 1×10^5 cells/l) in a mixture of equal parts of Eagles-Dulbecco and RPMI-1640²⁰ (Flow Laboratories Ltd) containing penicillin and streptomycin (100 $\mu\text{g/ml}$), buffered with bicarbonate. Cell viability of starting cultures was 75-90% by the trypan blue exclusion test. The HOM-2 cell line was established by Epstein-Barr virus transformation of peripheral blood lymphocytes obtained from two siblings homozygous at the LA and FOUR LA-A loci¹⁹; the cells are B type, as they contain the Epstein-Barr virus nuclear antigen,²¹ which is expressed exclusively on B cells.²² The CCRF-CEM cells were derived from a patient with acute lymphoblastic leukaemia¹⁸; they are therefore of tumour origin and have T-cell properties.^{21 23 24}

Retroplacental serum, which is composed mainly of intervillous blood plus decidual and placental interstitial fluid, was collected as described²⁵ during elective caesarean sections.

Control cultures contained 10% v/v swine serum (heat inactivated, 56°C, 30 min) and spermine (4 mmol/l; 5 ml/l; final concentration 20 $\mu\text{mol/l}$). Experimental cultures contained 1.25%, 2.5%, or 5% retroplacental serum (plus swine serum to 10%) with or without spermine. Cultures were incubated for 48 hours in a humidified atmosphere of 5% carbon dioxide in air. ^3H -thymidine (1 μl , 5 mg/l, specific activity 2 Ci/mmol; TRA 120 (Radiochemical Centre, Amersham) diluted with cold thymidine) was added to each culture two hours before harvesting.²⁶

Results are expressed as mean counts per minute over all cell concentrations for each treatment; calculation of counts per minute per 10^4 cells gave similar results. Probabilities were calculated by using Student's t test. Inhibition was considered significant when the ^3H -thymidine uptake of treated cells was less than 80% of control values. Serum polyamine oxidase activity was estimated as described^{27 28} and the results expressed as nmol/min/l.

Results

The table summarises the results. Interaction of spermine with maternal sera often resulted in a significant decrease in the uptake of ^3H -thymidine by cultured lymphocytes. In contrast, there was no difference between the two cell lines in uptake by control cultures. Retroplacental serum at 2.5% and 5% concentrations in the absence of spermine caused a significant decrease in the incorporation of radioactive material into T cells but, interestingly, no significant difference in uptake by B lymphocytes. Adding spermine to the cultures resulted in a significant decrease in the rate of proliferation of the B-cell line but had less effect on the T cells. The lowest concentration of serum (1.25%) had no significant effect in either combination. Inhibition of uptake of ^3H -thymidine occurred only when the serum polyamine oxidase activity exceeded 200 nmol/min/l (figure). The polyamine oxidase activity in peripheral maternal blood serum approached this level only near term.¹⁶

Discussion

Interaction of polyamine oxidase and substrate is reported to result in G_1 -phase arrest and not S-phase (DNA synthesis) arrest of cell proliferation.²⁹ Since we used non-synchronous cultures, cells were incubated with polyamine oxidase and substrate for 48 hours (equivalent to three average cell cycles) to increase the sensitivity of the assay. The difference in the effect of retroplacental serum on the two cell lines in the absence of exogenous spermine might possibly be due to the different

Division of Perinatal Medicine, Clinical Research Centre, Harrow, Middlesex, HA1 3UJ

DAVID M L MORGAN, MPHIL, chief research officer

GEORGE ILLEI, MD, Wellcome research fellow (present address: Markusovszky Teaching Hospital, Szombathely, Hungary.)

Effect of retroplacental serum on ^3H -thymidine uptake of lymphoid cell lines in culture. (Results expressed as means $\pm$ SEM)

Treatment	T (CCRF-CEM) cells			B (HOM-2) cells		
	No of observations	Counts per minute	p	No of observations	Counts per minute	p
Control (10% swine serum, 20 μmol spermine/l)	45	153.0 $\pm$ 12.2		45	152.0 $\pm$ 13.52	
Retroplacental serum:						
1.25%	9	163.1 $\pm$ 25.0	>0.5	9	208.1 $\pm$ 41.7	<0.02
2.5%	9	107.1 $\pm$ 21.6	<0.01	9	188.6 $\pm$ 33.9	>0.1
5.0%	9	68.4 $\pm$ 26.9	<0.01	9	118.6 $\pm$ 21.9	>0.1
Retroplacental serum and 20 μmol spermine/l:						
1.25%	9	138.5 $\pm$ 21.0	>0.5	9	118.3 $\pm$ 19.7	>0.5
2.5%	9	108.3 $\pm$ 25.3	<0.01	9	106.7 $\pm$ 23.6	<0.05
5.0%	9	88.6 $\pm$ 29.5	<0.01	9	91.1 $\pm$ 26.7	<0.01

Inhibition of lymphocyte proliferation in relation to polyamine oxidase activity of medium. Inhibition considered significant if ^3H -thymidine uptake of treated cells less than 80% of that of controls.

origins of the cells. CCRF-CEM cells are of tumour origin, and malignant lymphoblastic cells contain substantially higher concentrations of spermine than non-malignant lymphoid cells.^{30, 31} About 10-25% of the cells were dead at the start of culturing, and therefore the spermine concentration in the medium might have been sufficient for inhibition to occur without the addition of exogenous polyamine. Alternatively, T and B cells may possibly have different sensitivities to the products of the interaction of polyamine oxidase and polyamines, and this requires clarification.

It might be argued that the inhibition was caused by some factor(s) other than polyamine oxidase present in the retroplacental serum.¹² Our preliminary findings that a partially purified polyamine oxidase preparation similarly inhibited incorporation of ^3H -thymidine, however, indicate that the effect is unlikely to be due to factors other than the enzyme.

Our observations support the suggestion^{11, 12} that the products of the interaction of polyamines and polyamine oxidase through an inhibitory action on lymphoproliferation might "inhibit immune reactivity in a general way and thus represent a natural immunoregulatory agent."¹² Interestingly, Gunga *et al*³² and Porta *et al*³³ have shown that human placenta is rich in polyamines. Our data^{16, 17} indicate that polyamine oxidase activity increases during pregnancy and reaches remarkably high levels in the intervillous circulation, where the first and closest contact between fetal and maternal surfaces takes place. On the basis of our findings we suggest that the depressant effect of the interaction between polyamine oxidase and polyamines on lymphocyte proliferation might operate in vivo in the placental bed and so represent a localised immunological barrier, thereby forming a "first-line" or emergency defence; thus it perhaps in part contributes to protecting the conceptus from potential maternal immunological onslaught.

Since the mature placenta consists of a syncytial arrangement of trophoblasts that cannot divide, the cytostatic G_1 -phase

arrest of cell proliferation due to interaction of polyamine oxidase and substrate²⁹ should not itself result in damage to the placenta. Although polyamine oxidase is probably synthesised in the placenta or decidua¹⁷ and found in maternal serum, interestingly, fetal cord sera showed no polyamine oxidase activity.¹⁷ Malfunction of the interaction of polyamine oxidase and substrate, or indeed of diamine oxidase and substrate, might be a factor in the symptomatology or aetiology of clinically important disorders of pregnancy (for example, pre-eclampsia and threatening or spontaneous abortion).^{15, 34} A possible inhibitor of "amine oxidase" in pregnancy sera³⁵ might also be implicated.

We wish to emphasise, however, that the polyamine oxidase activity found in peripheral maternal serum might well be insufficient to cause widespread suppression of lymphoproliferation, which accords with the general belief that there is no substantial reduction at least in non-specific maternal immunoreactivity due to pregnancy.

This study was supported by a grant to GI from the Wellcome Trust.

References

- Walker JS, Freeman CB, Harris R. Lymphocyte reactivity in pregnancy. *Br Med J* 1972;iii:469.
- Gatti RA, Yunis EJ, Good RA. Characterization of a serum inhibitor of MLC reactions. *Clin Exp Immunol* 1973;13:427-37.
- St Hill CA, Finn R, Denye V. Depression of cellular immunity in pregnancy due to a serum factor. *Br Med J* 1973;iii:513-4.
- Jones E, Curzen P, Gaugas JM. Suppressive activity of pregnancy plasma on the mixed lymphocyte reaction. *Br J Obstet Gynaecol* 1973;80:603-7.
- Figueredo MA, Palomino P, Ortiz F. Lymphocyte response to phytohaemagglutinin in the presence of serum from pregnant women: correlation with serum levels of α -fetoprotein. *Clin Exp Immunol* 1979;37:140-4.
- Carr MC, Stites DP, Fudenberg HH. Cellular immune aspects of the human fetal maternal relationship. II. In vitro response of gravida lymphocytes to phytohemagglutinin. *Cell Immunol* 1973;8:448-54.
- Murgita RA, Andersson LC, Sherman MS, Bennick H, Wigzell H. Effects of human alpha-fetoprotein on human B and T lymphocyte proliferation in vitro. *Clin Exp Immunol* 1978;33:347-56.
- Adcock EW, Teasdale F, August CS, *et al*. Human chorionic gonadotrophin: its possible role in maternal lymphocyte suppression. *Science* 1973;181:845-7.
- Masson RL, Delire M, Cambiaso CL. Circulating immune complexes in normal human pregnancy. *Nature* 1977;266:542-3.
- Cerni C, Tatra G, Bohn H. Immunosuppression by human placenta lactogen (HPL) and the pregnancy-specific beta-1-glycoprotein (SP-1). Inhibition of mitogen-induced lymphocyte transformation. *Arch Gynaecol* 1977;223:1-7.
- Allen JC, Smith CJ, Curry MC, Gaugas JM. Identification of a thymic inhibitor ("chalone") of lymphocyte transformation as a spermine complex. *Nature* 1977;267:623-5.
- Byrd WJ, Jacobs DM, Amos MS. Synthetic polyamines added to cultures containing bovine sera reversibly inhibit in vitro parameters of immunity. *Nature* 1977;267:621-3.
- Rijke EO, Ballicux RE. Is thymus-derived lymphocyte inhibitor a polyamine? *Nature* 1978;274:804-5.
- Tabor H, Tabor CW. Biosynthesis and metabolism of 1,4-diaminobutane, spermidine, spermine, and related amines. *Adv Enzymol* 1972;36:203-68.
- Gaugas JM, Curzen P. Polyamine interaction with pregnancy serum in suppression of lymphocyte transformation. *Lancet* 1978;ii:18-20.

- ¹⁶ Illei G, Morgan DML. Polyamine oxidase activity in human pregnancy serum. *Br J Obstet Gynaecol* 1979;**86**:878-81.
- ¹⁷ Illei G, Morgan DML. The distribution of polyamine-oxidase activity in the fetomaternal compartments. *Br J Obstet Gynaecol* 1979;**86**:873-7.
- ¹⁸ Foley GE, Lazarus H, Farber S, Uzman BG, Boone RA, McCarthy RE. Continuous culture of human lymphoblasts from peripheral blood of a child with acute leukaemia. *Cancer* 1965;**18**:522-9.
- ¹⁹ Sly WS, Sekkon GS, Kennett R, Bodmer WF, Bodmer J. Permanent lymphoid lines from genetically marked lymphocytes: success with lymphocytes recovered from frozen storage. *Tissue Antigens* 1976;**7**:165-72.
- ²⁰ Morton HJ. Survey of commercially available tissue culture media. *In Vitro* 1970;**6**:89-108.
- ²¹ Kaplan J, Shope RC, Peterson WD. Epstein-Barr virus-negative human malignant T-cell lines. *J Exp Med* 1974;**139**:1070-76.
- ²² Ross G. Identification of human lymphocyte subpopulations by surface marker analysis. *Blood* 1979;**53**:799-811.
- ²³ Kaplan J, Mastrangelo R, Peterson WD. Childhood lymphoblastic lymphoma, a cancer of thymus-derived lymphocytes. *Cancer Res* 1974;**34**:521-5.
- ²⁴ Uzman BG, Foley GE, Farber S, Lazarus H. Morphologic variations in human leukaemic lymphoblasts (CCRF-CEM cells) after long-term culture and exposure to chemotherapeutic agents. *Cancer* 1966;**19**:1725-42.
- ²⁵ Klopfer A, Hughes G. Placental secretion of oestrogens and protein hormones. *Arch Gynaecol* 1978;**225**:171-7.
- ²⁶ O'Brien J, Knight S, Quick NA, Moore EH, Platt AS. A simple technique for harvesting lymphocytes cultured in Terasaki plates. *J Immunol Methods* 1979;**27**:219-23.
- ²⁷ Morgan DML, Ferluga J, Allison AC. Polyamine oxidase and macrophage function. In: Gaugas JM, ed. *Polyamines in biomedical research*. Chichester: John Wiley (in press).
- ²⁸ Morgan DML, Illei G. Radiochemical estimation of polyamine oxidase in serum and tissue in pregnancy. *Biochem Med* (in press).
- ²⁹ Gaugas JM, Dewey DL. Evidence for serum binding of oxidised spermine and its potent G₁-phase arrest of cell proliferation. *Br J Cancer* 1979;**39**:548-57.
- ³⁰ Rennert OM, Lawson DL, Skukla JB, Miale TD. Cerebrospinal fluid polyamine monitoring in central nervous system leukaemia. *Clin Chim Acta* 1977;**75**:365-9.
- ³¹ Rennert OM, Miale T, Shukla J, Frias J. Polyamine concentrations in bone marrow aspirates of children with leukaemia and other malignancies. *Blood* 1976;**47**:695-701.
- ³² Gunga CK, Sheth AR, Gunaga KP, Rao SS, Purandane MC. Polyamines in human placenta. *Indian J Biochem Biophys* 1972;**9**:272-4.
- ³³ Ponta R, Servillo L, Abbruzzese A, Pietra GD. Automated chromatographic analysis of human placenta polyamines. *Biochem Med* 1978;**19**:143-7.
- ³⁴ Southren AL, Kobayaski Y, Weingold AB, et al. Serial plasma diamine oxidase (DAO) assays in first and second trimester complications of pregnancy. *Am J Obstet Gynecol* 1966;**96**:502-10.
- ³⁵ Southren AL, Kobayaski Y, Carmody Y, et al. Serial measurements of plasma diamine oxidase (DAO) during normal human pregnancy: an improved method. Evidence for the presence of a circulating DAO inhibitor. *Am J Obstet Gynecol* 1966;**95**:615-20.

(Accepted 5 February 1980)

Idiopathic carpal tunnel syndrome caused by carpal stenosis

S DEKEL, T PAPAIOANNOU, G RUSHWORTH, R COATES

Summary and conclusions

Computed tomography was used to measure the cross-sectional area of the carpal canals in normal controls of both sexes and in women with idiopathic carpal tunnel syndrome. The women controls had significantly smaller carpal canals than the men controls both proximally and distally. In the patients both the proximal and distal cross-sectional areas were significantly reduced compared with the women controls. The measurements showed that carpal canal stenosis is associated with idiopathic carpal tunnel syndrome, narrowing of the canal is bilateral in patients who have unilateral symptoms, and narrowing is greater in the proximal carpal canal. There was no correlation between age and the size of the canal.

The difference in the size of the carpal canal between normal men and women might explain the tendency of women to develop carpal tunnel syndrome. The lack of correlation between age and the size of the canal suggests that stenosis of the carpal canal is inherited rather than acquired. Symptoms arise only later in life, when degenerative changes in the content or the walls of the carpal canal compete with the median nerve for space and its function becomes impaired by compression.

Introduction

The carpal tunnel syndrome is the most common peripheral nerve lesion and may be produced by several different conditions. In general, any pathological process that reduces the cross-sectional area of the carpal canal or swells its contents will compress the median nerve and produce the clinical manifestations of the syndrome. The carpal tunnel syndrome is well documented after bone fractures near the wrist,¹ dislocation of the carpal bones,² and soft-tissue tumours.³ In about half of the patients with the condition, however, the cause cannot be determined. Such patients with idiopathic carpal tunnel syndrome are often women approaching the menopause.⁴ It has been suggested⁵ that the idiopathic carpal tunnel syndrome is the result of anatomical narrowing of the carpal canal in women combined with oedema.

The aim of our study was to use computed tomography to measure the transverse dimensions of the carpal canal in patients with idiopathic carpal tunnel syndrome and in normal men and women, and to compare the results in the three groups.

Methods

The normal subjects comprised 19 women (mean age 39) and 14 men (mean age 42). We also studied 26 women with idiopathic carpal tunnel syndrome, in whom the diagnosis was based on the history and results of clinical examination and of electrophysiological studies on the median, ulnar, and radial nerves of the hands (details of these measurements will be reported separately).

Altogether we studied 42 hands: 16 patients had the syndrome in both hands and 10 in only one. Patients who might have one of the recognised diseases associated with carpal tunnel syndrome were excluded. The following complaints were associated with the condition: nocturnal pain (40 hands), paraesthesiae (40), pain in the hand (24), clumsiness (23), and pain in the forearm (21). The syndrome was

Nuffield Department of Orthopaedic Surgery, Nuffield Orthopaedic Centre, and University Department of Clinical Neurology, Churchill Hospital, Oxford

S DEKEL, MD, clinical lecturer in orthopaedic surgery

T PAPAIOANNOU, MD, research fellow

G RUSHWORTH, DM, university lecturer in clinical neurophysiology

R COATES, FRCS, clinical lecturer in orthopaedic surgery