MESANGIAL CELLS DERIVED FROM RAT GLOMERULI PRODUCE AN ERYTHROPOIESIS STIMULATING FACTOR IN CELL CULTURE

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1. Introduction

Erythropoietin (Ep) is a glycoprotein that stimulates the production of red blood cells. In response to hypoxia, the plasma level of Ep increases up to 2 or 3 orders of magnitude [1]. The increase is absent if nephrectomized animals are exposed to hypoxia [2,3].

This indicates that the kidney is essential for the biosynthesis of Ep. Within the kidney, Ep production has been associated with the glomerular region [4–7]. However, it is still unclear which of the cells constituting the glomerulus or its immediate vicinity are involved in the biosynthesis of Ep. Therefore, we have cultured the 2 types of cells which can be derived reproducibly from glomerular outgrowths, namely epithelial and mesangial cells [8–11]. We found that the culture medium of mesangial cells derived from rat glomeruli contained an erythropoietic activity which could not be distinguished from asialo-Ep. This activity was significantly higher in the medium of cells maintained at lower oxygen concentrations.

2. Materials and methods

2.1. *Cell culture*

Glomeruli from male Sprague Dawley rats (70–100 g body wt) were prepared under sterile conditions according to the sieving-technique in [12]. In brief, the kidneys were first flushed free of blood as in [13]. Renal cortical tissue was removed and consecutively passed through polyamide screens (Verseidag, Kempen) with pore sizes of 100 μm and 180 μm onto a screen of 50 μm pore size. Preparations of glomeruli from the 50 μm screen were confirmed under phase contrast microscopy to be free of contaminating single cells and tubular or arteriolar attachment. Aliquots of the glomeruli were placed in 75 cm^2 Falcon tissue flasks (Falcon Plastics) with 15 ml of RPMI 1640 (Boehringer, Mannheim), to which 10% fetal bovine serum (Boehringer, Mannheim), 100 IU/ml penicillin, 100 μg/ml streptomycin and 0.66 IU/ml insulin had been added as suggested in [14]. The flasks were incubated at 37°C in a humidified atmosphere containing either 20% or 3.5% O_2, 5% CO_2, balance N_2, using O_2- and CO_2-controlled incubators (Heraeus, Hanau). The medium was changed every third day.

Glomerular cell outgrowths were subcultured after treatment with 0.025 g/dl trypsin on day 6 and day 21 after the first inoculation, respectively. Subcultures obtained on day 6 are considered to derive from epithelial cells and subcultures from day 21 to derive from mesangial cells [11]. The culture medium was collected for a total of 3 weeks, concentrated 10 times on Amicon YM10 membranes and its erythropoietic activity measured as below.

2.2. *Erythropoietin assay*

The erythropoietic activity of the culture medium was determined using the fetal mouse liver cell assay for Ep. We determined the colony formation of erythroid precursor cells (CFU-e) obtained from 13 day fetal mouse livers. The test procedure was essentially that in [15] except that fetal bovine serum was replaced by a serum-free incubation mixture [16]. The latter modification was used to exclude effects of growth factors possibly present in fetal bovine serum. Ep standard curves were obtained with Ep, which had been pre-calibrated against the International Standard Preparation B in the polycythemic mouse assay for Ep.

In accordance with established procedures [15] we

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have expressed the erythropoietic activity of the unknown sample as the percentage of the maximal number of colonies which was obtained with standard Ep.

In some instances, the erythropoietic activity of the culture medium was determined by bioassay in vivo in exhypoxic polycythemic mice [17]. In this assay the incorporation of ^{59}Fe into newly formed erythrocytes is determined as a measure for the erythropoietic activity of the sample.

Further characterisation of the erythropoietic activity from the culture media included the effect of anti-Ep obtained from immunized rabbits on the erythropoietic activity [18], heat stability [1,19], and binding to DEAE-cellulose at pH 4.5 [20] and to wheat germ lectin [21].

3. Results and discussion

Table 1 shows that glomerular cell outgrowths, subcultured at day 6 (epithelial cells according to [11,22]) dit not produce significant erythropoietic activity when compared with control medium, either at 20% O_2 or 3.5% O_2. By contrast, cells subcultured at day 21 (mesangial cells according to [11,22]) released into the medium a factor that stimulated erythroid colony formation from fetal mouse liver cells. The colony-stimulating effect was significantly more pronounced when the cells had been maintained at 3.5% O_2 than at 20% O_2. From the dose-response curves obtained with standard Ep we estimated the erythropoietic activity to be 15 mU/ml and ~50 mU/ml in the medium from cell cultures grown at 20% O_2 and 3.5% O_2, respectively.

The erythropoietic activity from the culture medium was bound to DEAE-cellulose at pH 4.5. It could be eluted from the column with buffer of high pH and high ionic strength. The same chromatographic behaviour was reported for human urinary Ep [20]. Furthermore, the activity was found to be heat stable (100°C for 5 min) at pH 5.5. Again, this property is also found with plasma or urinary Ep [1,19]. Noteworthily, when the culture medium was first chromatographed on DEAE-cellulose and then treated with anti-Ep, the colony-stimulating effect almost disappeared (fig.1 left).

Chromatography was also done with wheat germ lectin, which is known to bind Ep mainly via *N*-acetylglucosamine residues [21,23]. Here, the bound material from the culture media which could be eluted with the disaccharide *N,N*-diacetylchitobiose exhibited all of the colony-stimulating activity whilst the unbound fraction contained none. Addition of anti-Ep to the fraction which could be eluted with *N,N*-diacetylchitobiose greatly inhibited the colony-stimulating activity (fig.1, right).

All these results are compatible with the hypothesis that the erythropoietically active substance from the culture media is in fact Ep. However, in several in vivo experiments in which the incorporation of ^{59}Fe was measured in vivo in polycythemic mice [17], no

Table 1
Effect of 3-week-pools of culture medium on the growth of erythroid precursor cells (CFU-e) expressed as percentage of maximal number of colonies

Oxygen	Control	Subculture: day 6 (epithelial cells)	Subculture: day 21 (mesangial cells)
20%	43 ± 4	33 ± 5	62 ± 2
3.5%	43 ± 4	36 ± 4	98 ± 8

Data represent mean ± SEM (n = 3)

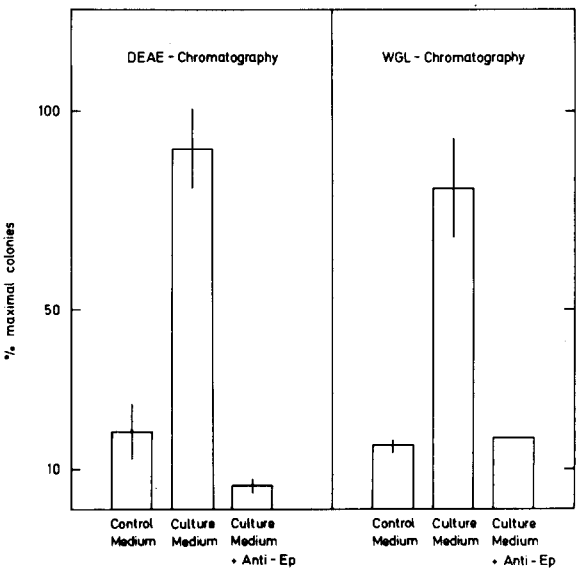

Fig.1. The effect of anti-erythropoietin (anti-Ep) on the colony stimulating effect of culture medium subjected to DEAE and wheat germ lectin (WGL) chromatography. Controls were made from freshly prepared incubation medium. Data are given as the percentage of maximal number of colonies (mean ± SEM, n = 3). Standard Ep (0.3 IU/ml) yielded 79 ± 2% of maximal colony number (2700 ± 280/10^5 nucleated liver cells, n = 7).

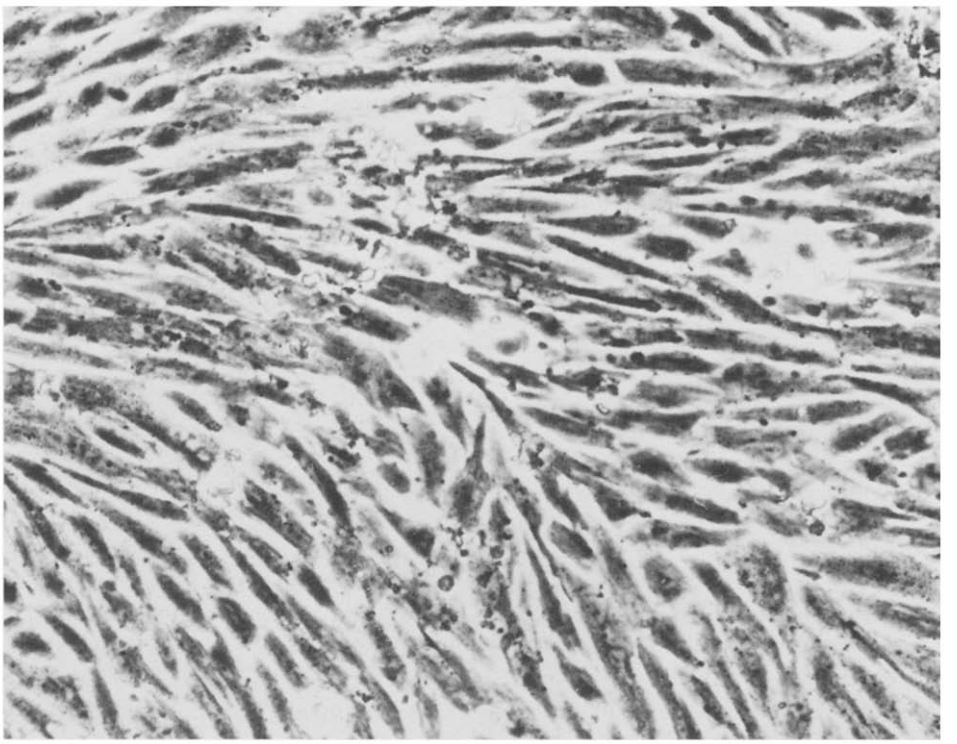

Fig.2. Phase micrograph of cells subcultured at day 21 from glomerular outgrowths. Magnification (200 ×).

enhancement of the ^{59}Fe incorporation was observed. This discrepancy can be most easily explained if one assumes that the erythropoietic factor which is active in vitro but not in vivo bears a reduced number of sialic acid residues. The absence of even a small percentage of sialic acid residues of Ep [26] as well as of other glycoproteins [24,25] leads to a rapid removal from the circulation whilst the in vitro activity is not affected [27].

We have performed additional experiments with Ep partly purified from the plasma of hypoxic rats which was treated with 0.004 IU/ml of neuraminidase for 16 h [23]. After the treatment, the Ep had lost its in vivo activity but retained all the properties of the erythropoietic activity from the above culture media.

We could not detect significant neuraminidase activity in the incubation medium after several days of culture. Why the cells in culture produce Ep that is not fully sialated, is being further investigated in this laboratory.

The type of cells which we obtained from subcultures of outgrowths from rat glomeruli at day 21 are elongated and grow in multilayers. This seems to be characteristic for mesangial cells but not for epithelial derived cells which grow in contact-inhibited monolayers [9,10,28]. A typical phase micrograph of the cells elaborating the erythropoietic activity above is shown in fig.2. These cells closely resemble the ones identified as mesangial cells in fig.1C of [28].

Finally, we wish to emphasize that the erythropoietic activity in the culture medium of these cells was found in a total of 9 independent glomerular-derived cell cultures and can therefore be regarded as a highly reproducible feature of renal mesangial cells in culture.

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References

[1] Erslev, A. J., Caro, J., Kansu, E. and Silver, R. (1980) Brit. J. Haematol. 45, 65–72.
[2] Jacobson, L. O., Goldwasser, E., Fried, W. and Plzak, L. (1957) Nature 179, 633–634.
[3] Schooley, J. C. and Mahlmann, L. J. (1972) Blood 40, 662–670.
[4] Fisher, J. W., Taylor, G. and Porteous, D. D. (1965) Nature 205, 611–612.
[5] Busuttil, R. W., Roh, B. L. and Fisher, J. W. (1972) Acta Haematol. 47, 238–242.
[6] Gruber, D. F., Zucali, J. R., Wleklinski, J., La Russa, V. and Mirand, E. A. (1977) Exp. Haematol. 5, 399–407.
[7] Burlington, H. and Cronkite, E. P. (1972) Proc. Natl. Acad. Sci. USA 12, 3547–3550.
[8] Holdsworth, S. R., Glasgow, E. M., Thomson, N. M. and Atkins, R. C. (1978) J. Pathol. 126, 231–237.
[9] Ishikawa, Y., Wada, T. and Sakaguchi, H. (1980) Am. J. Pathol. 100, 779–790.
[10] Scheinmann, J. J. and Fish, A. J. (1978) Am. J. Pathol. 92, 125–146.
[11] Foidart, J. B., Dechenne, C. A., Mahieu, P., Creutz, C. and Mey, J. (1979) Invest. Cell. Pathol. 2, 15–26.
[12] Krakower, C. A. and Greenspon, S. A. (1954) Arch. Pathol. 51, 629–639.
[13] Curthoys, N. P. and Bellemann, P. (1979) Exp. Cell. Res. 121, 31–49.
[14] Kreisberg, J. I., Hoover, R. L. and Karnowsky, M. J. (1978) Kidney Int. 14, 21–30.
[15] Rich, I. N. and Kubanek, B. (1979) J. Embryol. Exp. Morph. 50, 57–74.
[16] Iscove, N. N., Guilbert, L. J. and Weymann, C. (1980) Exp. Cell. Res. 126, 121–126.
[17] Cotes, P. M. and Bangham, D. R. (1961) Nature 191, 1065–1067.
[18] Jelkmann, W., Brookins, J. and Fisher, J. W. (1979) Proc. Soc. Exp. Biol. Med. 162, 65–70.
[19] Dorado, M., Langton, A. A. and Espada, J. (1971) Biochem. Med. 5, 195–204.
[20] Goldwasser, E., White, W. F. and Taylor, B. (1962) Biochim. Biophys. Acta 64, 487–496.
[21] Spivak, J. L., Small, D., Shaper, J. H. and Hollenberg, M. D. (1978) Blood 52, 1178–1188.
[22] Sraer, J., Foidart, J., Chansel, D. and Mahieu, P. (1979) FEBS Lett. 104, 420–424.
[23] Jelkmann, W. and Bauer, C. (1982) Pflügers Arch. in press.
[24] Morell, A. G., Gregoriadis, G., Sheinberg, I. H., Hickman, J. and Ashwell, G. (1971) J. Biol. Chem. 246, 1461–1467.
[25] Pricer, W. E. jr and Ashwell, G. (1971) J. Biol. Chem. 246, 4825–4833.
[26] Lukowsky, W. A. and Painter, R. H. (1972) Can. J. Biochem. 50, 909–917.
[27] Goldwasser, E. and Kung, C. K. H. (1968) Ann. NY Acad. Sci. 149, 49–53.
[28] Striker, G. E., Killen, P. D. and Farin, F. M. (1980) Transplantation Proc. 12/3 (suppl. 1) 88–99.