research paper
haematologica 2002; 87:465-471
http://www.haematologica.ws/2002_05/465.htm

Angiogenic activity of rat mast cells in the chick embryo chorioallantoic membrane is down-regulated by treatment with recombinant human α-2a interferon and partly mediated by fibroblast growth factor-2

DOMENICO RIBATTI,* ENRICO CRIVELLATO,°
LUIGI CANDUSSIO,# ANGELO VACCA,@ BEATRICE NICO,*
VINCENZO BENAGIANO,* LUISA RONCALI,*
FRANCO DAMMACCO@
*Department of Human Anatomy and Histology; @Department of Biomedical Sciences and Human Oncology, University of Bari Medical School, Bari; °Department of Medical and Morphological Research, Section of Anatomy, University of Udine Medical School, Udine; #Department of Biomedical Sciences, University of Trieste; Italy

Background and Objectives. Many data suggest that the density of mast cells (MC) is strongly correlated with the extent of both normal and pathologic angiogenesis, such as the vessel formation that occurs in chronic inflammatory diseases and tumors. We have previously demonstrated that isolated MC and their secretory granules, but not degranulated MC, induce an angiogenic response in the chick embryo chorioallantoic membrane (CAM) assay.

Design and Methods. The aim of this study was to investigate whether pre-treatment of MC with an anti-angiogenic molecule, namely recombinant human interferon-α2a (rhIFN-α2a), reduced the angiogenic activity of their conditioned media (CM) in the CAM assay.

Results. Our data indicate that rhIFN-α2a at 500-1000 IU is able to reduce the angiogenic activity of CM significantly. When MC were treated with rhIFN-α2a at 25-250 IU they retained their angiogenic activity. Addition of anti-fibroblast growth factor-2 (FGF-2) antibodies (but not anti-vascular endothelial growth factor) substantially reduced the angiogenic activity of CM treated with suboptimal concentrations of rhIFN-α2a.

Interpretation and Conclusions. FGF-2 may be the main angiogenic factor secreted by MC and higher concentrations of rhIFN-α2a possibly inhibit angiogenesis by blocking the actions of FGF-2 produced by MC. Finally, the morphologic features of MC treated with rhIFN-α2a, characterized by an atypical secretory pathway, are compatible with a slow release of the angiogenic cytokines stored in MC granules.

Key words: angiogenesis; anti-angiogenesis; chorioallantoic membrane; interferon-α; mast cells.

Correspondence: Prof. Domenico Ribatti, Department of Human Anatomy and Histology, piazza Giulio Cesare 11, Policlinico, 70124 Bari, Italy. Phone: international +39.080.5478240.
Fax: international +39.080.5478310. E-mail:ribatti@anatomia.uniba.it

Many data suggest that the density of mast cells (MC) is highly correlated with the extent of both normal and pathologic angiogenesis, such as the vessel formation that occurs in chronic inflammatory diseases and tumors.[1,2] MC release a variety of factors known to enhance angiogenic phenotype, namely heparin,[3] histamine,[4] and tryptase,[5,6] and several polypeptide growth factors, including fibroblast growth factor-2 (FGF-2), vascular endothelial growth factor (VEGF), transforming growth factor β (TGF-β), tumor necrosis factor α (TNF-α) and interleukin 8 (IL-8).[7-12]

We have previously demonstrated that isolated MC and their secretory granules, but not degranulated MC, induce an angiogenic response in the chick embryo chorioallantoic membrane (CAM) assay.[13] Addition of anti-FGF-2 or anti-VEGF antibodies reduced the angiogenic response induced by MC and their secretory granules and when antibodies were added together, their effect was additive. This finding demonstrated the principal role played by these two cytokines in the angiogenic response thus induced.

Interferons (IFNs) are a family consisting of three major natural glycoproteins with species specificity: leukocyte-derived IFN-α, fibroblast-derived IFN-β and immune-cell-produced IFN-γ. Systemic therapy with human recombinant IFN-α (rhIFN-α 2a) produces anti-angiogenic effects in vascular tumors, including hemangioma,[14-17] Kaposi's sarcoma,[18,19] melanoma,[20] basal and squamous cell carcinomas[21] and bladder carcinoma.[22] Regression of a recurrent giant tumor of the mandible was obtained by rhIFN-α2a administration at a low dose per day for 1 year.[23]

We have previously demonstrated an anti-angiogenic activity of rhIFN-α2a in the CAM[24] and the purpose of this study was to see whether treatment of conditioned medium of MC with rhIFN-α2a reduced its angiogenic activity in the CAM.

Design and Methods

Mast cell collection

MC were isolated from Wistar rats (Charles River, Como, Italy) as previously described.[25] Briefly, animals were anaesthetized with ether and killed by cervical dislocation. Their abdomens were massaged for 30 sec and the peritoneal cavity was then exposed through a ventral incision. After intraperitoneal injection of 10-20 mL of buffered saline solution (BSA: containing 154 mM NaCl, 2.7 mM KCl, 0.68 mM $CaCl_2$, 10 mM Na_2HPO_4, 10 mM KH_2PO_4, 1 g/L bovine serum albumin), adjusted to pH 7.2, cells were sedimented by centrifugation at 150 × g for 10 min. The supernatant was removed and cells were resuspended in buffered medium. MC were purified by a density gradient procedure according to Lagunoff and Rickard;[26] their purity in the final preparation, as determined by staining with toluidine blue, was more than 90% (about 95-97% in some experiments). The contaminating cell population observed by electron microscopy consisted of middle-sized or large non-granular lymphocytes and, to a lesser degree, macrophages.

Mast cell incubation with conditioned media containing rhIFN-α2a

Purified MC were suspended in sterile RPMI-1640 (5×10^5 cells/mL), with an osmolality of 300±8 mosm/kg. The medium was buffered to pH 7.3 with 10 mM HEPES, supplemented with 10% fetal calf serum and 1% penicillin/streptomycin. Cells were maintained for 24 h at 37°C in culture medium alone or incubated with various amounts of rhIFN-α2a (Roferon-A; Roche, Grenzach, Wyhlen, Germany) (25, 250, 500, 1000 U/mL), diluted stepwise with RPMI-1640. Conditioned media (CM) were collected, clarified by centrifugation, concentrated ten times with Mr 3000 cut-off centrifugal concentrators (Centriplus, Amicon, Beverly, MA, USA) and stored at –70°C until use. Experiments were carried out in triplicate from 3 different CM collections.

Morphometric quantitative evaluation of MC exocytosis after incubation with rhIFN-α 2a

For quantitative evaluation of ultrastructural changes induced by rhIFN-α2a, purified MC were incubated with 500 or 1000 U/mL rhIFN-α2a or with the medium alone for 24 h, as described above. They were then fixed with 2.5% glutaraldehyde in phosphate buffer (PB) 0.1 M, pH 7.4 for 1 h at 4°C, postfixed with 1% osmium tetroxide in PB 0.1 M, for 2 h at 4°C. The pellets were then dehydrated in a graded ethanol series, immersed in propylene oxide and embedded in Epon 812. Ultrathin sections were cut with a diamond knife on a Reichert Jung Ultracut E ultratome, doubly stained with uranyl acetate and lead citrate and observed in a Philips CM 12 electron microscope at 80 KV. Randomly sectioned grids taken from each block of uniformly distributed cells were examined. Exocytosis was evaluated in groups of randomly selected MC as follows: 1) focal exocytosis (one to three secretory granules in the cell); 2) mild exocytosis (about less than 30% of secretory granules); 3) moderate exocytosis (30 to 60% of the granules); 4) diffuse exocytosis (more than 60% of the granules); 5) atypical exocytosis (one or two dilated, non-fused granular cavities with partially eroded or dissolved granular matrices).

CAM assay

Fertilized White Leghorn chicken eggs (20 for each treatment) were incubated at constant humidity and 37° C. On the third day of incubation, a square window was opened in the shell after removal of 2-3 mL of albumin so as to detach the developing CAM from the shell. The window was sealed with a glass of the same size, and the eggs were returned to the incubator.

In the first series of experiments, gelatin sponges (1 mm^3; Gelfoam, Upjohn Company, Kalamazoo, USA) were placed on the CAM at day 8 and, according to Ribatti *et al.*,[27] adsorbed with 3 μL of CM of MC alone or treated with 25, 250, 500 and 1000 IU rhIFN-α2a. In some experiments, to the CM treated with 25 and 250 IU rhIFN-α2a, were added 400 ng of rabbit polyclonal anti-FGF-2 or anti-VEGF antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA), or pre-immune rabbit serum (Dako, Glostrup, Denmark), used as a negative control. CAMs implanted with sponges containing recombinant FGF-2 (R&D Systems, Abingdon, UK) were used as positive controls. At day 12, CAMs were photographed *in ovo*. All CAMs were then processed for light and electron microscopy. Briefly, the embryos and their membranes were fixed *in ovo* in 3% phosphate-buffered glutaraldehyde. The sponges and the underlying and immediately adjacent portions of CAM were dehydrated in serial alcohols, postfixed in 1% phosphate-buffered O_sO_4 and embedded in Epon 812. One micron semithin and ultrathin sections were cut on a LKB V ultramicrotome according to a plane parallel to the surface of CAM. The semithin sections were stained with a 0.5% aqueous solution

Table 1. Morphometric quantitative data of exocytosis in mast cells incubated with 500 and 1,000 IU/mL rhIFN-α2a for 24 h.

Morphologic features	*500 U/mL (134 cells)*	*1,000 U/mL (143 cells)*	*Control (182 cells)*
Resting cells	78	71	163
Focal exocytosis	11	15	–
Mild exocytosis	2	3	–
Moderate exocytosis	–	–	14
Diffuse exocytosis	–	–	5
Atypical exocytosis	43	54	–

of toluidine blue (Merck, Darmstadt, Germany) and observed under a Leitz-Dialux 20 light microscope (Leitz, Wetzlar, Germany). The ultrathin sections were stained with uranyl acetate followed by lead citrate and examined in a Philips CM 12 electron microscope at 80 KV.

The angiogenic response was assessed histologically by a planimetric method of *point counting*.[27] Briefly, every third section in 30 serial slides from each specimen was analyzed under a 144-point mesh inserted into the eyepiece of the photomicroscope. The total number of intersection points occupied by transversally cut vessels (3-10 μm diameter) was counted at ×250 both inside the sponge and in 6 randomly chosen fields per each section. Mean values ± SD were determined for each analysis. Vascular density was defined as the final mean number of the occupied points. The results from the experiments were compared using a one-way analysis of variance (ANOVA). *p* values less than 0.5 were considered to be statistically significant.

Results

Ultrastructural analysis and morphometric quantitative evaluation of MC exocytosis after incubation with rhIFN-α2a

In control samples, about 90% MC (Table 1) appeared as resting, unstimulated cells (Figure 1A). The remaining 10% (Table 1) displayed ultrastructural features compatible with mild to moderate exocytosis (Figure 1B). These MC displayed the common findings of granule fusion, intracytoplasmic channel formation and granule opening to the cell surface. A small proportion of these cells (2-3% of total control MC, Table 1) underwent typical generalized exocytosis, accompanied by extrusion of altered granular matrices (Figure 1C).

When MC were incubated with 500 or 1,000 U/mL rhIFN-α2a for 24 h, about 50-60% cells (Table 1) presented the ultrastructural features of resting, unstimulated cells (Figure 2A). Some MC (~10%, Table 1), however, displayed focal exocytosis, which was characterized either by opening of

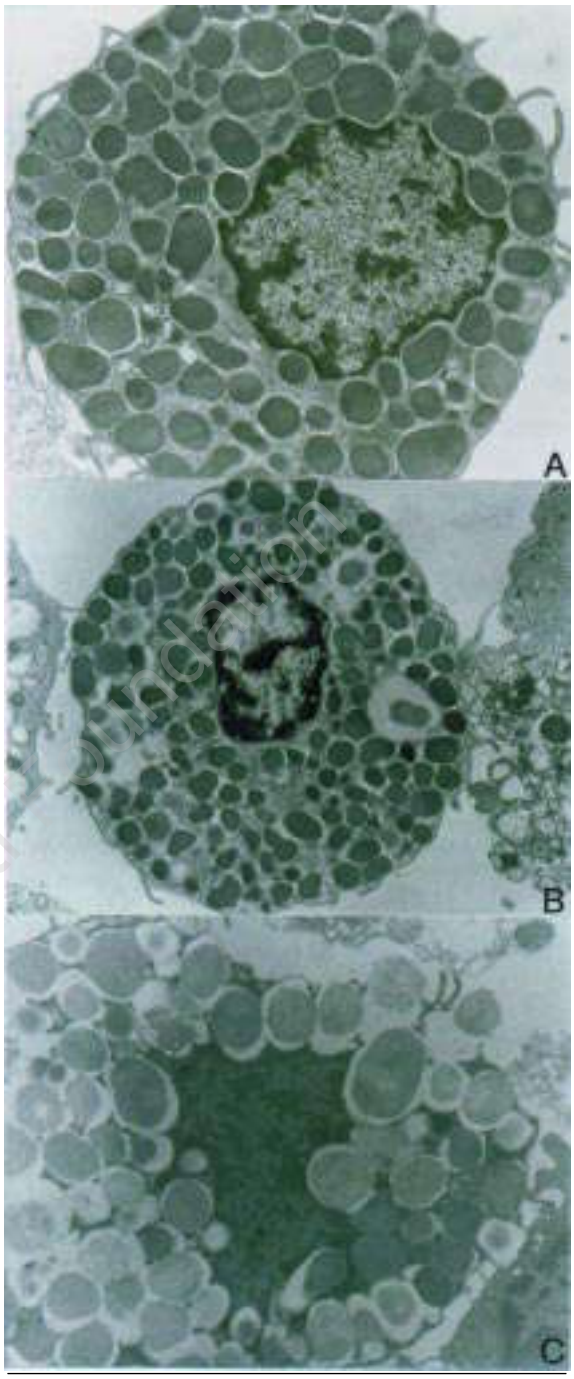

Figure 1. Ultrastructural features of control mast cells; (A) 90% of cells show features of resting, unstimulated cells; (B) 10% of cells are characterized by granule fusion and intracytoplasmic channel formation, compatible with a process of mild to moderate exocytosis; (C) 2-3% of cells present a generalized exocytosis, accompained by extrusion of altered granular matrices. Original magnification: A-C, ×9,000.

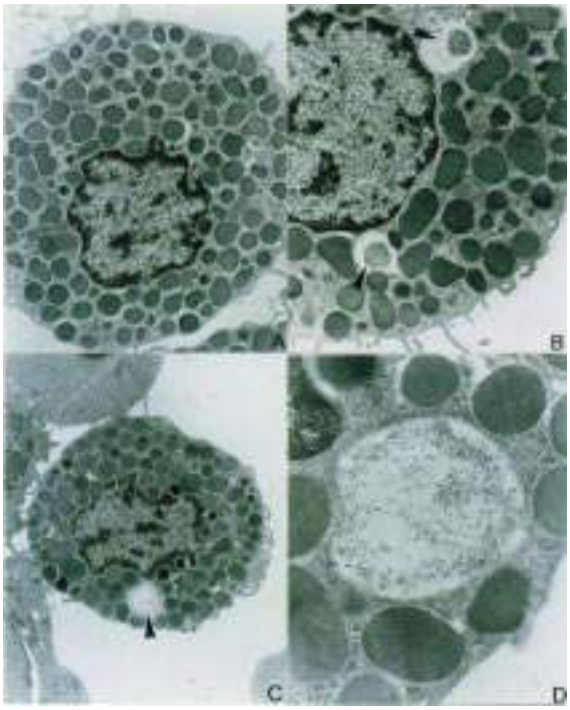

Figure 2. Ultrastructural features of mast cells treated with 500 IU/mL rhIFN-α 2a for 24 h; (A) 50-60% of cells present the characteristics of resting cells; (B) 10% of cells exhibit fusion of 2-3 granules into a small secretory channel (arrowheads), expression of a focal exocytosis; (C) 30-40% of cells contain, in their cytoplasm, expanded, not fused granular cavities containing partially dissolved or eroded granular matrices (arrowhead in C, and at higher magnification in D). Original magnification: A, C, ×9,000; B, ×13,000; D, × 18,000.

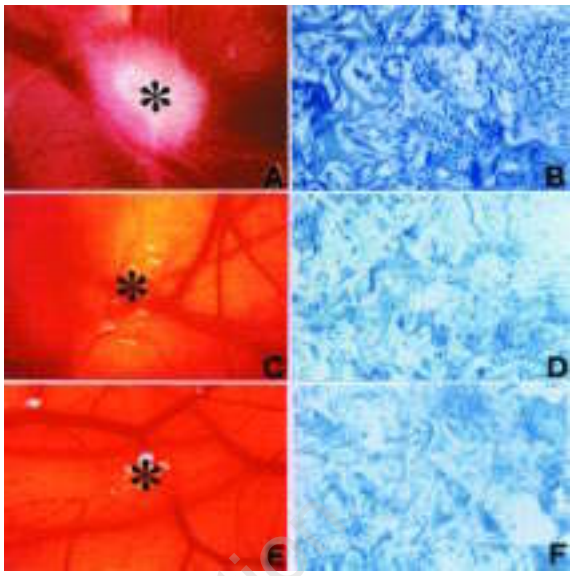

Figure 3. In A and B, effects of conditioned media (CM) of untreated mast cells on CAM vascularization. (A) Macroscopically, CM induce an angiogenic response in the form of allantoic vessels that develop radially towards the implant (asterisk) in a *spoked-wheel pattern*. (B) Microscopically, a highly vascularized tissue is recognizable among the sponge trabeculae. In C-F, effect on the angiogenic response induced by treatment with rhIFN-α 2a at 500 IU (C, D) and of the incubation of the CM treated with rhIFN-α 2a at 25 IU with a neutralizing anti-FGF-2 antibody (E, F). In both conditions, macroscopically (C, E) and microscopically (D, F) few blood vessels are detectable around and, respectively, inside the sponge. Original magnification: A, C, E, ×50; B, D, F, 250.

single granules to the cell surface or by fusion of 2-3 granules into small secretory channels (Figure 2B). The most common feature of rhIFN-α2a-treated MC was an atypical exocytosis observed in 30 to 40% cells (Table 1). Within the cytoplasm of these MC there were one or two expanded, not fused granular cavities containing partially dissolved or eroded granular matrices (Figures 2C, D). Serial sectioning demonstrated that these granules did not fuse with other granules, nor open to the cell exterior. On the other hand, the remaining cytoplasmic granules showed no sign of activation. Co-existence of focal exocytosis with large, non-fused granule formation was occasionally observed in the same cell. True exocytosis involving the whole cell granular repertoire was never observed.

CM of MC treated with rhIFN-α2a at 25 and 250 IU do not exert an anti-angiogenic activity in the CAM assay

Macroscopic inspection of incubation day 12 showed that the gelatin sponges loaded with untreated CM were surrounded by allantoic vessels that developed radially towards them in a *spoked wheel* pattern (Figure 3A). The angiogenic response was comparable to that induced by FGF-2 (positive control) (*not shown*). Microscopically, in the sponges loaded with untreated CM, a highly vascularized tissue was recognizable among the sponge trabeculae as newly formed blood vessels within an abundant network of collagen fibers (Figure 3B). Vessel density was higher at the periphery than in the center of the sponge, and at the boundary between the sponge and the CAM mesenchyme there were numerous capillaries that frequently pierced the sponge. Macroscopic and microscopic observations were confirmed by the morphometric evaluation using the planimetric method of *point counting* (Table 2).

Treatment with rhIFN-α2a at 25 and 250 IU was unable to inhibit the angiogenic response induced by the untreated CM (Table 2). Otherwise, when the gelatin sponges were loaded with CM treated

Table 2. Effects on CAM vascularization of mast cell conditioned media (CM)°.

Treatment	*No. of intersection points (Mean ±SD)*	*Microvessel density (%)*
CM	30±2	20.8
CM + 25 IU rhIFN-α2a	28±3	19.4
CM + 25 IU rhIFN-α2a + anti-FGF-2 ab	14±3^	9.7^
CM + 25 IU rhIFN-α2a + anti-VEGF ab	25±4	17.4
CM + 250 IU rhIFN-α2a	25±3	17.4
CM + 250 IU rhIFN-α2a + anti-FGF-2 ab	12±4^	8.3^
CM + 250 IU rhIFN-α2a + anti-VEGF ab	25±5	17.3
CM + 500 IU rhIFN-α2a	15±2*	10.4*
CM + 1000 IU rhIFN-α2a	13±3*	9.0*
CM + pre-immune serum	28±4	19.4
FGF-2	32±4	22.2

*°Mast cells were maintained for 24 h at 37°C in culture medium alone or added to 25, 250, 500 and 1,000 IU rhIFN-α2a and CM were tested in the CAM assay. In some experiments, CM were tested in the presence of neutralizing anti-FGF-2, anti-VEGF antibodies or pre-inmmune rabbit serum, used as negative controls. *Statistically different from CM alone (ANOVA p < 0.05). ^Statistically different from CM + 25/250 IU rhIFN-α2a (ANOVA p < 0.05).*

with rhIFN-α2a at 500 and 1,000 IU, very few blood vessels were macroscopically detectable around the sponge (Figure 3C) and microscopically there were few vessels among the trabeculae (Figure 3D) and the boundary. Macroscopic and microscopic observations were confirmed by morphometric evaluation (Table 2).

When a neutralizing anti-FGF-2 antibody was added to the CM treated with rhIFN-α2a at 25 and 250 IU, the angiogenic activity was significantly reduced; the morphologic findings (Figures 3E, 3F) and morphometric evaluation (Table 2) were similar to those recognizable when CM treated with rhIFN-α2a at 500 and 1000 IU were tested. Addition of neutralizing anti-VEGF antibody did not reduced the angiogenic acitivity of the CM (Table 2).

Discussion

Here we show that when CM of rat MC are treated with rhIFN-α2a at 500 and 1000 IU, the angiogenic activity of untreated CM tested in the chick embryo CAM assay is significantly reduced and that the most typical feature of rhIFN-α2a-treated MC is the presence in their cytoplasm of one or two expanded, not fused granular cavities containing partially dissolved or eroded granular matrices. Serial sectioning examination demonstrates that these granules do not fuse with other granules and do not open to the cell exterior. On the other hand, the remaining cytoplasmic granules show no sign of activation. These morphologic features might be suggestive of a slow release of the granule contents and may explain the fewer angiogenic factors in the CM of rhIFN-α2a-treated MC, compared to the untreated MC. Slow release of soluble MC mediators, unlike Ig-E mediated massive degranulation during the immediate hypersensitivity reaction, also occurs in delayed hypersensitivity reactions, chronic inflammatory processes, such as rheumatoid arthritis, and tumors, such as multiple myeloma.[28,29]

FGF-2 is a cytokine involved in the angiogenic activity of MC and rhIFN-α2a inhibits angiogenesis by blocking the actions of FGF-2 produced by MC

Qu *et al.*[30] have demonstrated that FGF-2 immunoreactivity is located in the cytoplasmic granules of MC in several human tissues, and that immunogold particle localization for FGF-2 is also present in their extruded granules. They have also demonstrated immunohistochemically that FGF-2 is located in most MC from normal skin and lung and in tissue samples displaying fibrosis, hyperplasia, and neovascularization.[7]

We have previously demonstrated that isolated MC and their secretory granules, but not degranulated MC induce an angiogenic response in the CAM assay.[13] The addition of anti-FGF-2 or anti-VEGF antibodies reduced the angiogenic response of both MC and their secretory granules and when the two antibodies were added together, their effect was additive.[13] This illustrates their principal role in induction of the angiogenic response induced by isolated MC and their secretory granules in the CAM vasculature.

IFN-α inhibits *in vitro* FGF-2-induced endothelial cell migration and proliferation[31,32] and systemic therapy with rhINF-α2a produces anti-angiogenic effects in vascular tumors, including hemangioma,[14,17] Kaposi's sarcoma,[18,19] melanoma,[20] basal and squamous cell carcinomas[21] and bladder carcinoma.[22] These tumors have been shown to produce the high levels of FGF-2 often detectable in the urine or serum of the patients.[33-35] IFN-α decreased the expression of FGF-2 mRNA and protein in human renal cell cancer as well as in human bladder, prostate, colon and breast carcinoma cells.[36] Systemic administration of rhIFN-α2a decreased both the *in vivo* expression of FGF-2 and blood vessel density, and inhibited tumor growth of a human bladder carcinoma implanted orthotopically in nude mice.[37]

In this study we show that the angiogenic activity of the CM of MC treated with rhIFN-α2a at concentrations unable to inhibit their angiogenic activity was significantly reduced when neutralizing anti-FGF-2 antibodies were added to these CM, while anti-VEGF antibodies were unable to reduce the angiogenic activity of the CM. The specificity

of inhibition of FGF-2 following treatment with rhIFN-α2a is also confirmed by previous data demonstrating that in CAM assay that anti-VEGF antibody blocks the angiogenic effect of exogenous VEGF[38] and that anti-FGF-2 antibody blocks the effect of FGF-2.[39]

It is conceivable to hypothesize that rhIFN-α2a may interfere with FGF-2 through other mechanisms such as physiologic antagonism, rather than only its release in the CM. In fact, our preliminary data indicate that when rhIFN-α2a is added to the CM after its collection and before its addition to the CAM, it retains its anti-angiogenic activity. This is in accord with previous data showing that IFN-α directly inhibits, in several tumor cell lines, mRNA and protein production of FGF-2 by a mechanism independent of its antiproliferative effect.[36]

In conclusion, our data indicate that rhIFN-α2a at 500-1,000 IU significantly reduces the angiogenic activity of CM collected from MC and tested in the CAM assay. It is conceivable that this angiogenic activity is prevalently mediated by FGF-2 contained in the MC secretory granules and released in their CM. In fact, CM pre-treated with lower concentrations of rhIFN-α2a, retain their angiogenic activity in the CAM assay and this activity is significantly reduced by addition of anti-FGF-2 antibodies. Lastly, the morphologic features of MC treated with rhIFN-α2a are characterized by an atypical secretory pathway and are compatible with slow release of the angiogenic cytokines stored in MC granules, such as FGF-2.

Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

Contributions and Acknowledgments

DR was primarily responsible for this work, from conception to submitted manuscript. The remaining authors, listed according to a criterion of decreasing individual contribution to this article, contributed equally to the laboratory experiments and statistical analyses. The last author had a major role as senior author in interpreting the data and preparing the article.

Funding

This work was supported in part by a grant from Ministero dell'Università e della Ricerca Scientifica e Tecnologica (MURST, local funds), Rome, Italy, to DR, from Associazione Italiana per la Lotta al Neuroblastoma, Genoa, Italy, to DR, and by a grant from Associazione Italiana per la Ricerca sul Cancro (AIRC), Milan, Italy, to DR and AV.

References

1. Meininger CJ, Zetter BR. Mast cells and angiogenesis. Semin Cancer Biol 1992; 3:73-9.
2. Norrby K, Wooley D. Role of mast cells in mitogenesis and angiogenesis in normal tissues and tumor tissue. Advan Biosci 1993; 89:71-115.
3. Ribatti D, Roncali L, Nico B, Bertossi M. Effects of exogenous heparin on the vasculogenesis of the chorioallantoic membrane. Acta Anat 1987; 130:257-63.
4. Sorbo J, Jakobsson A, Norrby K. Mast cell histamine is angiogenic through receptors for histamine1 and histamine2. Int J Exp Pathol 1994; 75:43-50.
5. Blair RJ, Meng H, Marchese MJ, Ren S, Schwartz LB, Tonnesen MG, et al. Human mast cells stimulate vascular tube formation. Tryptase is a novel, potent angiogenic factor. J Clin Invest 1997; 99:2691-700.
6. Ribatti D, Vacca A, Marzullo A, Nico B, Ria R, Roncali L, et al. Angiogenesis and mast cell density with tryptase activity increase simultaneously with pathological progression in B-cell non-Hodgkin's lymphomas. Int J Cancer 2000; 85:171-5.
7. Qu Z, Liebler JM, Powers MR. Mast cells are a major source of basic fibroblast growth factor in chronic inflammation and cutaneous hemangioma. Am J Pathol 1995; 147:564-73.
8. Grutzkau A, Kruger-Krasagakes S, Kogel H, Moller A, Lippert U, Henz BM. Detection of intracellular interleukin-8 in human mast cells: flow cytometry as a guide for immunoelectron microscopy. J Histochem Cytochem 1997; 45:935-45.
9. Boesiger J, Tsai M, Maurer M, Yamaguchi M, Brown LF, Claffey KP, et al. Mast cells secrete vascular permeability factor/vascular endothelial growth factor and exhibit enhanced release after immunoglobulin E-dependent upregulation of Fcε receptor I expression. J Exp Med 1998; 188:1135-45.
10. Moller A, Henz BM, Grutzkau A, Lippert U, Aragane Y, Schwarz T, et al. Comparative cytokine gene expression: regulation and release by human mast cells. Immunology 1998; 93:289-95.
11. Yamada T, Sawatsubashi M, Yakushiji H, Itoh Y, Edakuni G, Mori M, et al. Localization of vascular endothelial growth factor in synovial membrane mast cells: examination with 'multi-labelling subtraction immunostaining'. Virchows Arch 1998; 433: 567-70.
12. Fan L, Iseki S. Immunohistochemical localization of vascular endothelial growth factor in the globule leukocyte/mucosal mast cells of the rat respiratory and digestive tracts. Histochem Cell Biol 1999; 111:13-21.
13. Ribatti D, Crivellato E, Candussio L, Nico B, Vacca A, Roncali L, et al. Mast cells and their secretory granules are angiogenic in the chick embryo chorioallantoic membrane. Clin Exp Allergy 2001; 31:602-8.
14. White CW, Sondheimer HM, Crouch EC, Wilson H, Fan LL. Treatment of pulmonary hemangiomatosis with recombinant interferon α-2a. N Engl J Med 1989; 320:1197-200.
15. Ezekowitz RA, Mulliken JB, Folkman J. Interferon α-2a therapy for life-threatening hemangiomas of infancy. N Engl J Med 1992; 326:1456-63.
16. Ricketts RR, Hatley RM, Corden BJ, Sabio H, Howell CG. Interferon α-2a for the treatment of complex heman-

giomas of infancy and childhood. Ann Surgery 1994; 219: 605-14.
17. Kuipper RA, Schellens JH, Blijham GH, Beijnen JH, Voest EE. Clinical research on antiangiogenic therapy. Pharmacol Res 1998: 37:1-16.
18. Real FX, Oettgen HF, Krown SE. Kaposi's sarcoma and the acquired immunodeficiency syndrome: treatment with high and low doses of recombinant leukocyte α interferon. J Clin Oncol 1986; 4:544-51.
19. Mitsuyasu RT. Interferon α in the treatment of AIDS-related Kaposi's sarcoma. Br J Haematol 1991; 79:69-73.
20. Legha SS. The role of interferon α in the treatment of metastatic melanoma. Semin Oncol 1997; 24:524-31.
21. Tucker SB. Interferon-α treatment of basal cell and squamous cell skin tumors. Cancer Bull 1993; 45:270-4.
22. Stadler WM, Kuzel TM, Raghavan D, Levine E, Vogelzang NJ, Roth B, et al. Metastatic bladder cancer: advances in treatment. Eur J Cancer 1997; 33 Suppl 1:S23-6.
23. Kaban LB, Mulliken JB, Ezekowitz RA, Ebb D, Smith PS, Folkman J. Antiangiogenic therapy of a giant cell tumor of the mandible with interferon α-2a. Pediatrics 1999; 103:1145-9.
24. Minischetti M, Vacca A, Ribatti D, Iurlaro M, Ria R, Pellegrino A, et al. TNP-470 and recombinant human interferon-α2a inhibit angiogenesis synergistically. Br J Haematol 2000; 109:829-37.
25. Crivellato E, Candussio L, De Corti G, Bartoli Klugmann F, Mallardi F. Adriamycin induces exocytosis in rat and beige mouse peritoneal mast cells: an ultrastructural, morphometric and biochemical study. J Submicrosc Cytol Pathol 1999; 31:279-86.
26. Lagunoff D, Rickard A. Methods for study of rat peritoneal mast cell secretion. In: Poisner AM, Trifarò JM, editors. In vitro methods for studying secretion. The secretory process. Amsterdam: Elsevier Science Publishers; 1987. p. 13-28.
27. Ribatti D, Gualandris A, Bastaki M, Vacca A, Iurlaro M, Roncali L, et al. New model for the study of angiogenesis and antiangiogenesis in the chick embryo chorioallantoic membrane: the gelatin sponge/chorioallantoic membrane assay. J Vasc Res 1997; 34:455-63.
28. Ribatti D, Contino, R, Tursi A. Do mast cells intervene in the vasoproliferative processes of the rheumatoid synovitis? J Submicrosc Cytol Pathol 1988; 20:635-7.
29. Ribatti D, Vacca A, Nico B, Quondamatteo F, Ria R, Minischetti M, et al. Bone marrow angiogenesis and mast cell density increase simultaneously with progression of human multiple myeloma. Br J Cancer 1999; 79:451-5.
30. Qu Z, Kayton RJ, Ahmadi P, Liebler JM, Powers MR, Planck SR, et al. Ultrastructural immunolocalization of basic fibroblast growth factor in mast cell secretory granules. Morphological evidence for bfgf release through degranulation. J Histochem Cytochem 1998; 46:1119-28.
31. Ruszczak Z, Detmar M, Imcke E, Orfanos CE. Effects of rIFN-α, β and γ on the morphology, proliferation, and cell surface antigen expression of human dermal microvascular endothelial cell in vitro. J Invest Dermatol 1990; 95:693-9.
32. Pepper MS, Vassalli JD, Wilks JW, Schweigerer RL, Orci L, Montesano R. Modulation of bovine microvascular endothelial cell proteolytic properties by inhibitors of angiogenesis. J Cell Biochem 1994; 55:419-34.
33. Nanus DM, Schmitz-Drager BJ, Motzer RJ, Lee AC, Vlamis V, Cordon-Cardo C, et al. Expression of basic fibroblast growth factor in primary human renal tumors: correlation with poor survival. J Natl Cancer Inst 1993; 85:1597-9.
34. Nguyen M, Watanabe H, Budson AE, Richie JP, Hayes DF, Folkman J. Elevated levels of angiogenic peptide, basic fibroblast growth factor, in the urine of patients with a wide spectrum of cancers. J Natl Cancer Inst 1994; 86: 356-61.
35. O'Brien TS, Smith K, Cranston D, Fuggle S, Bicknell R, Harris AL. Urinary basic fibroblast growth factor in patients with bladder cancer and benign prostatic hypertrophy. Br J Urol 1995; 76:311-4.
36. Singh RK, Gutman M, Bucana CD, Sanchez R, Llansa R, Fidler IJ. Interferons α and β down-regulate the expression of basic fibroblast growth factor in human carcinomas. Proc Natl Acad Sci USA 1995; 92:4562-6.
37. Dinney CP, Bielenberg DR, Perrotte P, Reich R, Eve BY, Bucana CD, et al. Inhibition of basic fibroblast growth factor expression, angiogenesis, and growth of human bladder carcinoma in mice by systemic interferon-α administration. Cancer Res 1998; 58:808-14.
38. Nakamura S, Murakami-Mori K, Rao N, Weich HA, Rajeev B. Vascular endothelial growth factor is a potent angiogenic factor in AIDS-associated Kaposi's sarcoma-derived spindle cells. J Immunol 1997; 158:4492-5001.
39. Ribatti D, Urbinati C, Nico B, Rusnati M, Roncali L, Presta M. Endogenous basic fibroblast growth factor is implicated in the vascularization of the chick embryo chorioallantoic membrane. Dev Biol 1995; 170:39-49.

Peer Review Outcomes

Manuscript processing

This manuscript was peer-reviewed by two external referees and by Professor Mario Cazzola, Editor-in-Chief. The final decision to accept this paper for publication was taken jointly by Prof. Cazzola and the Editors. Manuscript received November 6, 2001; accepted March 19, 2002.

What is already known on this topic

Mast cells have a role both in normal and pathologic angiogenesis.

What this study adds

Fibroblast growth factor-2 is involved in the angiogenic activity of mast cells and interferon α inhibits angiogenesis by blocking this cytokine.

Potential implications for clinical practice

Interferon α might be employed to inhibit angiogenesis specifically.

Mario Cazzola, Editor-in-Chief