Review

Neovascularisation and its role in the osteoarthritic process

SUMMARY In osteoarthritis angiogenesis is involved in the reinitiation of cartilage growth and mineralisation. A number of heparin binding protein growth factors have been proposed as angiogenic factors, but none of them is specific for microvessel cells. Another factor which is *specific for microvessel cells*, is of low molecular weight and non-protein has been called endothelial cell stimulating angiogenic factor (ESAF). ESAF has been found in significantly increased amounts in sera and synovial fluids of osteoarthritic patients and dogs. In addition to its angiogenic activity ESAF is able to activate neutral prometalloproteinases and to reactivate the active enzyme-inhibitor complex. The implication of these observations in the pathogenesis of osteoarthritis is discussed.

The view is now widely held that the osteoarthritic process is not solely a degenerative one. Rather, mechanical damage gives rise to some change in the nature of the articular cartilage, perhaps an altered cellular activity whose main purpose is regenerative but whose consequence is destructive. Conversely, it is arguable that altered cellular activity may also predispose the tissue to osteoarthritic changes.^{1–3} Possible contenders for such early change involve the calcification of cartilage,⁴ the formation of new bone,^{1 5} and the penetration of new blood vessels. Indeed it is difficult to separate bone neovascularisation and calcification as they normally occur together as linked processes. Implicit in this view of osteoarthritis (OA) is the idea that the reinitiation of cartilage growth and mineralisation are pre-requisites of the osteoarthritic process (PD Byers, RA Brown, unpublished data).^{1 4 6} This review examines the regulation and importance of neovascularisation (angiogenesis) as one component of that process.

Angiogenic factors

The importance of angiogenesis for tumour growth has been accepted for many years, in fact since the end of the last century,^{7 8} but the concept of its involvement in OA is new.^{9 10} The idea that a diffusible substance may be capable of inducing uncontrolled angiogenesis in the retina, leading to blindness, was originally proposed by Michaelson in 1948.¹¹ Judah Folkman coined the term 'tumour

angiogenesis and isolated a factor in very crude form from tumours, which he called 'tumour angiogenesis factor'.¹² Evidence that angiogenesis occurred abnormally in situations where no tumour was involved, such as the proliferative retinopathy of diabetes which Michaelson had studied and in the advancing pannus of the rheumatoid arthritic joint, could not be ignored, however.

In 1979 a low molecular weight angiogenic factor was isolated from tumour extracts. This factor, which is not a protein, was also found in human synovial fluid.¹³ Unlike tumour angiogenic factor, which at that time had a reported molecular weight of the order of 80 000 daltons, this material was very small with a molecular mass of less than 600 daltons. The low molecular weight factor was called 'endothelial cell stimulating angiogenesis factor (ESAF)'. Other angiogenic factors have been proposed, and the original tumour angiogenesis factor has been shown to contain a protein of molecular weight approximately 17 000 daltons, which was first thought to be a specific endothelial cell growth factor but which was later shown to be the same as the previously described fibroblast growth factor.^{14 15} This factor is, as its name implies, not specific to endothelial cells but is also able to cause proliferation of mesenchymal cells such as fibroblasts and smooth muscle cells. Fibroblast growth factor therefore cannot be considered as a true angiogenic factor. ESAF, on the other hand, is only mitogenic to microvessel endothelial cells and has no activity towards mesenchymal cells or even to

large vessel (aortic) endothelial cells.¹⁶ Recently, ESAF has also been shown to stimulate proliferation of pericytes (A Schor, personal communication).

In 1980 it was shown that two thirds of all synovial fluids from patients with OA contained amounts of ESAF sufficient to produce angiogenesis in an *in vivo* test system—namely, the chick chorioallantoic membrane.^{9–10} Only about 15% of synovial fluids from patients with rheumatoid arthritis (RA) gave similar results.

In that study no consideration was given as to whether these few patients with RA were also suffering from OA. Subsequently, radiographic examination of those patients with RA and increased levels of ESAF suggested that there is an association with the presence of osteophytes. In view of the neovascularisation observed in RA the relative absence of ESAF suggests that inflammatory angiogenesis may be controlled through a mechanism distinct from that involved in bone growth. More recent work has shown that angiogenesis of RA is stimulated by a population of macrophages which secrete tumour necrosis factor alpha.^{17–18} This factor has a molecular weight of 17 500 daltons and has been characterised and synthesised as a gene product. Macrophages are rare in OA, however, and even in inflammatory forms of OA such as crystal arthropathies the number of macrophages is far fewer than that found in rheumatoid arthritic joints.¹⁹

A remarkable property of ESAF is its ability to activate neutral prometalloproteinases: procollagenase, progelatinase, and to a lesser extent prostromelysin (J B Weiss, S C-S Ng, and B McLaughlin, unpublished data).^{20–21} All these enzymes have a key role in the degradation of connective tissue, including cartilage. Controlling the active forms of these enzymes is tissue inhibitor of metalloproteinase, which interacts with them to form inactive complexes.²² Reactivation of these enzyme-inhibitor complexes has until now been considered to be a non-physiological proposition. Recent work, however, has shown that ESAF is also able to dissociate this enzyme-inhibitor complex (T Cawston and J B Weiss, unpublished data).²¹ These findings suggest that the role of ESAF in neovascularisation may be via a limited degradation of connective tissue, which would allow space for the new capillaries to penetrate.

Involvement of angiogenesis in OA

How does neovascularisation relate to joint changes in OA? The relation is identifiable in three processes—the growth of osteophytes,^{1–2–5–23} advance of the subchondral plate,^{4–6–24} and ‘remodelling’ of the joint.^{25–27} Each has been implicated in the

pathogenesis of OA. Classically, marginal osteophytes form when capillaries penetrate the subchondral plate and the deep zone of articular cartilage.² Endochondral ossification follows to produce new bone over the original cartilage. Clearly, in the early stages of the growth of peripheral osteophytes the barrier to neovascularisation presented by articular cartilage must be breached, at least locally. Cartilage contains inhibitors of both angiogenesis and metalloproteinases,^{28–32} which would need to be overcome in some way for this breach to occur. Osteophytes are normally present in osteoarthritic joints; exceptions such as ‘billiard ball hip’ are rare.³³ Advance of the subchondral plate by calcification of deep zone articular cartilage has been equated with the histological landmark termed ‘tide mark reduplication’.¹ Such encroaching calcification can give rise to thinning of the articular cartilage^{4–24} or to growth of the joint (P D Byers and R A Brown, unpublished data),⁶ depending on the cartilage response. In either case vascular invasion is an inevitable stage. Indeed, a failure of cartilage to produce the protease inhibitors which prevent neovascularisation has been put forward as a basis for the initiation of cartilage loss.³⁴ Renewal of cartilage calcification has been implicated as a change related to OA, on the basis of increased cartilage alkaline phosphatase activity²⁴ and accumulation of microcrystals and matrix vesicles.³⁵ Encroachment of the subchondral plate in adult joints has also been identified, by the presence of unremodelled cartilage islands, in bony trabeculae beneath this plate (P D Byers and R A Brown, unpublished data).⁶ Remodelling of the joint to produce an altered geometry probably involves remodelling of the subchondral plate region²⁷ as above. This is said to lead to increased congruency of the joint and impaired load carrying with eventual cartilage degeneration by mechanical failure.^{25–26}

Morphological features and *in vitro* observations

The morphological features of vascular invasion during endochondral ossification have been examined by a number of reviewers.^{1–2–36–37} Neovascularisation in the mammalian growth plate is the most dramatic example of this as the ordered synchronous invasion of capillaries into the avascular cartilage emphasises the degree of regulation. Clearly, the balance between stimulation and inhibition of angiogenesis is stark in this location. Invasive vessels penetrate the calcified cartilage as blind ended sprouts³⁸ consistent with the low oxygen tension of this region.³⁹ Microvascular invasion is along the chondrocyte columns, through the unmineralised transverse septae which are eroded by

perivascular cells at the tip of the sprout (R A Brown *et al*, unpublished data).^{40 41} Invasive perivascular cells normally degrade the cartilaginous transverse septae by insertion of numerous cell processes (R A Brown *et al*, unpublished data).⁴⁰ These processes presumably utilise proteolytic enzyme systems on their surface to achieve collagenolysis. Locally active collagenase is essential for this lysis and it is in activating this enzyme that ESAF is thought to be critical.⁴² The nature of the invasive perivascular cells is uncertain. Although they have been termed macrophage-like,⁴¹ our own observations suggest that they may be specialised endothelial cells (R A Brown *et al*, unpublished data), similar to those described in experimental angiogenesis.⁴³

In vitro experiments using calcifying cultures of growth plate chondrocytes suggested to us that ESAF is produced in the zones of hypertrophy and provisional mineralisation.⁴² As chondrocytes in such high density cultures begin to mineralise their cartilage matrix and become surrounded by calcific deposits they also release ESAF such that it appears in the medium between 24 and 48 hours after initiation of calcification. Suppression of mineralisation by inhibition of alkaline phosphatase reduces ESAF production, though stimulation of production is not directly dependent on alkaline phosphatase activity.⁴⁴ Production of ESAF in culture, then, suggested that ESAF from calcifying chondrocytes in vivo forms a diffusion gradient towards the capillary bed. Inactive collagenase produced by invasive endothelial cells^{45 46} (presumably around their cell processes, which lie within the cartilage septae) would be activated locally, resulting in controlled collagen degradation. Subsequently, both elements of the invading capillary sprout pass through the transverse septum, along the gradient of ESAF. Connective tissue breakdown may also occur around mature hypertrophic chondrocytes during hypertrophy,^{28 47} though recent work suggests that this is related more to connective tissue reorganisation than to resorption and cell degeneration.^{48 49} In this context it is interesting that collagenolytic enzymes are present in osteoarthritic cartilage.^{50 51}

The role of ESAF

ESAF activity, induced by sectioning the cruciate ligament, has been demonstrated in the synovial fluids of dogs with OA.⁵² The appearance of ESAF in the fluids was cyclical over a given time period—that is, its concentration rose and fell. Levels of ESAF did not correlate with the fluid volume or the numbers or types of cells present in the effusions. The cyclical rhythm of ESAF release may also occur in humans, and probably reflects the observation

that not all patients' fluids aspirated on different occasions gave the same result.¹⁰ Subsequent work with ESAF has shown that it is not only present in excess in the synovial fluid of patients with OA but also in the serum of patients with active OA.⁵³ In dogs, after sectioning of the cruciate ligaments, an increase in serum ESAF levels similar to that seen in their synovial fluid also occurs (D Bennett *et al*, unpublished data).⁵²

The consequence of ESAF accumulation in joint effusions is uncertain at present. Preliminary work using intra-articular injections of purified ESAF indicates that ESAF produces no gross macroscopic changes and no loss of joint function. This is perhaps not surprising as such a regulator must itself be controlled and inactivated, particularly in the body fluids. Indeed, most of the ESAF in serum is reversibly bound to macromolecular carriers in adults, though there is an excess of ESAF to carrier in fetal serum. Accumulation of ESAF in the synovial fluid and serum probably represents a stage in its clearance from the joint. Consequently, its accumulation would indicate past activity in the cartilage rather than a stimulus to new activity, or changes with pathological consequences.

If ESAF does indeed work through its ability to activate procollagenase and progelatinase, and thus to create a space for new blood vessels to move into the matrix, then it is not improbable that some inhibitors of collagenase and gelatinase could also be acting as inhibitors of ESAF. The tissue inhibitor of metalloproteinase, which is the major inhibitor so far described, is not capable of inhibiting the angiogenic activity of ESAF either in vivo or in vitro tests (unpublished observations). Specific inhibitors of angiogenesis are, however, present in cartilage and vitreous,^{31 32} and one of these (in vitreous) also inhibits collagenase. Platelet factor 4 is another example of an inhibitor of collagenase and angiogenesis.^{54 55} The presence in OA cartilage of a low molecular weight activator of collagenolytic enzymes, in transit diffusion from bone to synovial fluid, may be seen as a possible and indeed likely contributor to the degradative process in that condition. When the structure of ESAF is known it is not unlikely that analogues may have a therapeutic role.

In conclusion, the role of neovascularisation in the pathogenesis of OA is inevitably bound up with cartilage calcification and ossification. As evidence from other sources has implicated calcific changes and bone growth with joint degeneration it is perhaps not surprising that ESAF is also present. It is exciting that by analogy with growth plate neovascularisation it is possible to propose a mechanism by which cartilage lysis and capillary invasion

(and by implication calcification) are regulated through chondrocyte production of ESAF. There still remains the question of at which stage in the process of osteoarthritic joint degeneration neo-vascularisation is important—early, as a precipitating factor, or, later, during the extensive growth of new bone.

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References

- Sokoloff L. *The biology of degenerative joint disease*. Chicago: University of Chicago Press, 1969.
- Trueta J. Degenerative arthritis. In: *Studies of the development and decay of the human frame*. London: Heinemann, 1968.
- Howell D S, Woessner J F, Jimenez S, Seda H, Schumacher H R. A view on the pathogenesis of osteoarthritis. *Bull Rheum Dis* 1979; **29**: 996–1007.
- Ali S Y. New knowledge of osteoarthritis. *J Clin Pathol [Suppl] (R Coll Pathol)* 1978; (suppl 12): 191–9.
- Gilbertson E M M. Development of periarticular osteophytes in experimentally induced osteoarthritis in the dog. *Ann Rheum Dis* 1975; **34**: 12–25.
- Johnson L C. Morphologic analysis in pathology. In: Frost H M, ed. *Bone biodynamics*. London: Churchill, 1964: 543–654.
- Kregger L. On local alterations of the blood-vessels of tar painted mice. *Br J Exp Pathol* 1927; **8**: 465–76.
- Thiessen N W. The vascularity of benign and malignant lesions of the stomach. *Surg Gynecol Obstet* 1936; **63**: 149–56.
- Brown R A, Weiss J B, Tomlinson I W, Phillips P, Kumar S. Angiogenic factor from synovial fluid resembling that from tumours. *Lancet* 1980; **i**: 682–5.
- Brown R A, Tomlinson I W, Hill C R, Weiss J B, Phillips P, Kumar S. Relationship of angiogenesis factor in synovial fluid to various joint diseases. *Ann Rheum Dis* 1983; **42**: 301–7.
- Michaelson I C. The mode of development of the vascular system of the retina, with some observations on its significance for certain retinal diseases. *Trans Ophthalmol Soc UK* 1948; **68**: 137–80.
- Folkman J, Marler E, Abernathy C, Williams G. Isolation of a tumour factor responsible for angiogenesis. *J Exp Med* 1971; **133**: 275–88.
- Weiss J B, Brown R A, Kumar S, Phillips P. An angiogenic factor isolated from tumours: a potent low molecular weight compound. *Br J Cancer* 1979; **40**: 493–6.
- Klagsbrun M, Sasse J, Sullivan R, Smith J A. Human tumour cells synthesise an endothelial cell growth factor that is structurally related to basic FGF. *Proc Natl Acad Sci USA* 1986; **83**: 2448–52.
- Folkman J, Klagsbrun M. Angiogenic factors. *Science* 1987; **235**: 442–7.
- Schor A M, Schor S L, Weiss J B, Brown R A, Kumar S, Phillips P. Stimulation by a low molecular weight angiogenesis factor of capillary endothelial cells in culture. *Br J Cancer* 1980; **41**: 790–9.
- Leibovich S J, Polverini P J, Shepard H M, Wiseman D M, Shively Y, Nuseir N. Macrophage-induced angiogenesis is mediated by tumour necrosis factor α . *Nature* 1987; **329**: 630–2.
- Frater-Schroder M, Risau W, Hollman R, Gautschi P, Bohlen P. Tumour necrosis factor type α , a potent inhibitor of endothelial cell growth in vitro, is angiogenic in vivo. *Proc Natl Acad Sci USA* 1987; **84**: 5277–81.
- Naid Z M. Cytology of synovial fluids. *Acta Cytol (Baltimore)* 1973; **17**: 299–309.
- Weiss J B, Hill C R, Davis R J, McLaughlin B, Sedowofia K A, Brown R A. Activation of procollagenase by low molecular weight angiogenesis factor. *Biosci Rep* 1983; **3**: 171–7.
- Weiss J B, Odedra R, McLaughlin B. Low molecular mass angiogenic factors. In: Tsuchiya M, ed. *Microcirculation and update*. Vol 2. Amsterdam: Elsevier, 1987: 777–80.
- Welgus H G, Jeffrey J J, Eisen A Z, Roswit W T, Stricklin G P. Human skin fibroblast collagenase: interaction with substrate and inhibitor. *Coll Relat Res* 1985; **5**: 167–79.
- Byers P D, Contepomi C A, Farkas T A. A post mortem study of the hip joint. (2) Histological data for limited and progressive lesions. *Ann Rheum Dis* 1976; **35**: 114–21.
- Ali S Y, Bayliss M T. Enzymic changes in human osteoarthritic cartilage. In: Ali S Y, Elves M W, Leaback D H, eds. *Normal and osteoarthritic articular cartilage*. London: Institute of Orthopaedics, University of London, 1974.
- Bullough P G, Goodfellow J W, Greenwald A S, O'Connor J. Incongruent surfaces in the human hip joint. *Nature* 1968; **217**: 1290.
- Bullough P G. The geometry of diarthrodial joints, its physiological maintenance and the possible significance of age-related changes in geometry to load distribution and the development of osteoarthritis. *Clin Orthop* 1981; **156**: 61–6.
- Lanc L B, Villacin A, Bullough P G. The vascularity and remodelling of subchondral bone and calcified cartilage in adult human femoral and humeral heads. *J Bone Joint Surg [Br]* 1977; **59**: 272–9.
- Kuettner K E, Pauli B U. Vascularity of cartilage. In: Hall B K, ed. *Cartilage, structure, function and biochemistry*. Vol 1. New York: Academic Press, 1983: 287–312.
- Kuettner K E, Hilti J, Eisenstein R, Harper E. Collagenase inhibition by cationic protein derived from cartilage and aorta. *Biochem Biophys Res Commun* 1976; **72**: 40–6.
- Eisenstein R, Sorgente N, Soble L W, Miller A, Kuettner K E. Penetrability of explanted tissues by vascularized mesenchyme. *Am J Pathol* 1973; **73**: 765–76.
- Eisenstein R, Kuettner K E, Neopolitan C, Soble L W, Sorgente N. The resistance of certain tissues to invasion. III. Cartilage extracts inhibit the growth of fibroblasts and endothelial cells in culture. *Am J Pathol* 1975; **81**: 337–48.
- Taylor C M, Weiss J B. Partial purification of a 5.7K glycoprotein from bovine vitreous which inhibits both angiogenesis and collagenase activity. *Biochem Biophys Res Commun* 1987; **143**: 947–53.
- Sokoloff L. Osteoarthritis as a remodelling process. *J Rheumatol [Spec Issue]* 1987; **14**: 7–10.
- Stephens R, Ghosh P, Taylor T. The pathogenesis of osteoarthritis. *Med Hypotheses* 1979; **5**: 809–16.
- Ali S Y, Griffiths S. Formation of calcium phosphate crystals in normal and osteoarthritic cartilage. *Ann Rheum Dis* 1983; **42** (suppl): 45–8.
- Brookes M. *The vascular supply of bone*. London: Butterworth, 1971.
- Schenk R K. Basic histomorphology and physiology of skeletal growth. In: Weber B G, Brunner C H, Freuler F, eds. *Treatment of fractures in children and adolescents*. Berlin: Springer, 1980.

- 38 Arsenault A L. Microvascular organisation at the epiphyseal-metaphyseal junction of growing rats. *Journal of Bone Mineral Research* 1987; **2**: 143-9.
- 39 Brighton C T. The growth plate. *Orthop Clin North Am* 1984; **15**: 571-95.
- 40 Schenk R K, Wiener J, Spiro D. Fine structure aspects of vascular invasion of the tibial epiphyseal plate of growing rats. *Acta Anat (Basel)* 1968; **68**: 1-17.
- 41 Anderson C E, Parker J. Invasion and resorption in endochondral ossification. *J Bone Joint Surg [Am]* 1966; **48**: 899-914.
- 42 Brown R A, Taylor C, McLaughlin B, McFarland C D, Weiss J B, Ali S Y. Epiphyseal growth plate cartilage and chondrocytes in mineralising cultures produce a low molecular mass angiogenic procollagenase activator. *Bone Mineral* 1987; **3**: 143-58.
- 43 Ausprunk D, Folkman J. Migration and proliferation of endothelial cells in preformed and newly formed blood vessels during angiogenesis. *Microvasc Res* 1977; **14**: 53-65.
- 44 Brown R A, McFarland C D, Ali S Y, McLaughlin B, Taylor C M, Weiss J B. Alkaline phosphatase substrates and in vitro production of the angiogenic factor, ESAF, by calcifying cartilage. *Br J Rheumatol* 1987; **26** (suppl 2): 65.
- 45 Herron G S, Banda M J, Clark E J, Gavrilovic J, Werb Z. Secretion of metalloproteinases by stimulated capillary endothelial cells. *J Biol Chem* 1986; **261**: 2814-8.
- 46 Moscatelli D A, Rifkin B D, Jaffe E A. Production of latent collagenase by human umbilical vein endothelial cells in response to angiogenic preparations. *Exp Cell Res* 1985; **156**: 379-90.
- 47 Dean D D, Muniz O E, Berman I, *et al.* Localisation of collagenase in the growth plate of rachitic rats. *J Clin Invest* 1985; **76**: 716-22.
- 48 Schenk R K, Eggli P S, Hunziker E B. Articular cartilage morphology. In: Kuettner K E, Schleyerbach R, Hascall Y C, eds. *Articular cartilage biochemistry*. New York: Raven Press, 1986: 3-22.
- 49 Hunziker E B, Schenk R K, Cruz-Orive L-M. Quantitation of chondrocyte performance in growth plate cartilage during longitudinal bone growth. *J Bone Joint Surg [Am]* 1987; **69**: 162-73.
- 50 Ehrlich M G, Houle P A, Vigliani G, Mankin H J. Correlation between articular cartilage collagenase activity and osteoarthritis. *Arthritis Rheum* 1978; **21**: 761-6.
- 51 Ehrlich M G, Armstrong A L, Treadwell B Y, Mankin H J. Role of proteases in the pathogenesis of osteoarthritis. *J Rheumatol [Spec Issue]* 1987; **14**: 30-2.
- 52 Taylor C M, Weiss J B, McLaughlin B, Grennan D M, Bennett D. Cyclic variation in ESAF levels in dogs with experimental osteoarthritis. *Int J Microcirc Clin Exp* 1987; **5**: 389.
- 53 Weiss J B, Murphy J, McLaughlin B, Mitchell W, Grennan D. Measurement in serum from patients with rheumatic disease of levels of a non-enzymatic activator of latent metalloproteinase, which is also angiogenic. *Br J Rheumatol* 1987; **26** (suppl 2): 65.
- 54 Hiti-Harper J, Wohl H, Harper E. Platelet factor 4: an inhibitor of collagenase. *Science* 1978; **199**: 991-2.
- 55 Folkman J, Taylor S, Spillberg C. The role of heparin in angiogenesis. In: Nugent J, O'Connor M, eds. *Development of the vascular system*. London: Pitman, 1983: 132-49. (Ciba Foundation Symposium, No 100.)