Osteoclasts and haematopoietic stem cells in developing human bones

THE stimulating paper by Scheven *et al.*[1] concluded that murine osteoclast-forming capacity rises with increasing haematopoietic stem cell purity when cocultured with preosteoclast-free embryonic long bones. However, a growth-factor-like effect of introduced stem cells should be considered.

In 1982 we called attention to the fact that the appearance of osteoclasts in May-Grünwald-Giemsa-stained smears and serial sections from different bones in the course of human haematopoietic development precedes the onset of haematopoiesis in each bone by about two weeks[2]. This accords with the development of the haematopoietic stroma. So if osteoclasts are derived from haematopoietic stem cells their presence in the marrow could be regarded as a very early 'footprint' of these otherwise undetectable stem cells.

Osteoclasts, formerly believed to be of the monocyte/macrophage lineage could be derived from a marrow stem cell unrelated to the stem cell for other blood cells. There could also still be some doubt about the nature of giant cells appearing during long-term bone marrow culture, that is, in long-term culture of newborn rabbit bone marrow giant cells have been characterized as macrophage polykaryons rather than osteoclastic giant cells[3].

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SCHEVEN AND NIJWEIDE REPLY—Because the haematopoietic cell populations used in our study were not 100% pure, we cannot completely exclude the possibility that other stem cells, not identical to the haematopoietic stem cell, were co-purified and gave rise to osteoclast formation. However, the fact that interleukin-3 pretreatment of the stem cell preparations stimulated osteoclast development, as well as formation of granulocytes and macrophages, strengthen the possibility that the haematopoietic stem cell is also the progenitor for osteoclasts.

The first appearance of osteoclasts in developing embryonic bones should not be regarded as a footprint of the presence of haematopoietic stem cells. Osteoclasts differentiate in the cartilagenous models of long bones either from committed haematogeneous progenitor cells seeded in the perichondrium or from haematopoietic multipotent progenitors committed to differentiate into osteoclasts under the influence of the long bone model. Osteoclasts excavate a marrow cavity in the calcified hypertrophic cartilage area, creating room for stroma formation. Osteoclasts and stromal cells are together responsible for the establishment of a microenvironment for homing and maintenance of haematopoietic stem cells, which are transported via the blood stream from the early haematopoietic organ (liver). Considering this sequence of events it is not surprising that the osteoclasts appear before the onset of bone marrow haematopoiesis.

Finally, we are convinced that the multinucleated cells formed during co-culture with periostless metatarsal bones are true osteoclasts and not macrophage polykaryons. The multinucleated cells have morphological, enzyme-histochemical (tartrate-resistant acid phosphatase) and functional (resorption) characteristics of osteoclasts. Furthermore, in several nonpublished experiments we have used quail bone marrow as exogenous source of osteoclast progenitors. The multinucleated cells developed during co-culture with fetal mouse long bones could be recognized by their specific chromatin organization as quail cells, and by their reaction with osteoclast-specific monoclonal antibodies[1] as osteoclasts.

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Interactions of N-CAM with heparin-like molecules

COLE *ET AL.*[1] tried to answer the question of whether the binding of heparan sulphate to the neural cell adhesion molecule, N-CAM, is also required for cell adhesion. They showed that the binding of retinal probe cells to retinal cell monolayers was inhibited by heparin, a substance similar to heparan sulphate; but not by chondroitin sulphate. Monoclonal antibodies that recognized two different domains on N-CAM, the homophilic and heparin-binding domains, inhibited cell adhesion. The heparin-binding domain isolated from N-CAM by selective proteolysis also inhibited cell-cell adhesion when bound to the probe cells.

Heparan sulphate is one of the major constituents of the extracellular matrix, is found on cell surfaces, and throughout the mammalian body either in its free form or bound covalently or electrostatically by ionic forces to proteins. Heparin is found in small amounts in the mammalian system and its biosynthesis, functions and degradation involve enzyme systems different from those involved in the metabolism of heparan sulphate. The distinctiveness of the two metabolic systems is expressed in human pathology as, for example, Sanfilippo disease type A, characterized by the lack of heparan sulphamidase. Only changes in heparan sulphate metabolism can be detected; heparin metabolism is unaffected. This is cited to show the biological and biochemical differences between those two compounds. The structural differences between the two acid glycosaminoglycans are well documented. The major difference is the degree of sulphation and the length of chains. As the degree of sulphation is responsible for the binding mechanisms of this highly negatively charged polyanion, heparin should have been used rather as a control substance in addition to the use of heparan sulphate in the protein interaction studies.

The use of heparitinase, which like heparan sulphate, is available, could have helped or could be suggested for confirmation of the data obtained.

Chondroitin sulphate is not a good control in our opinion as it is completely different from heparan sulphate and heparin: the sulphate in this compound is bound to the polysaccharide skeleton by *O*-sulphation and not, as in heparan sulphate and heparin by *N*-sulphation. Thus, even if chain length and degree of sulphation of the glycosaminoglycan were comparable, the unknown charge distribution of 'chondroitin sulphate mixed isomers'[1] could not be compared. The reactivity of heparan sulphate (and heparin) with proteins is enormous. This has been shown by several authors. Gelman and coworkers studied the glycosaminoglycan-protein interaction using circular dichroism[2] and Doyle and associates published the interaction between collagen and acid glycosaminoglycans indicating that sulphated glycosaminoglycans were binding (nonspecifically) to clustered, basic, positively charged amino-acid residues[3].

Laminin, an intrinsic glomerular basement membrane protein which is involved in cell-basement membrane interactions, and fibronectin interact with heparan sulphate and heparin[4,5]. So does collagen type IV and the native glomerular basement membrane, containing covalently linked heparan sulphate-proteoglycan[6]. Thus, heparan sulphate seems to be a highly reactive substance resembling other compounds (such as laminin, actin and fibronectin) with 'glue-activity'.

In our hands this acid-glycosaminoglycan reacts with commercially available polylysine as a marker substance for