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Historical Review

PAUL EHRLICH'S DOCTORAL THESIS: A MILESTONE IN THE STUDY OF MAST CELLS

The history of mast cell research begins with a name and a date. The name is that of Paul Ehrlich; the date is the 17 June 1878. That day, the 24-year-old medical student from Strehlen (Schlesien) presented his doctoral thesis at the Medical Faculty of Leipzig University. The title of his dissertation was 'Contribution to the theory and practice of histological dyes' ('Beiträge zur Theorie und Praxis der histologischen Färbung') (Ehrlich, 1878). This work is a beautiful and admirable example of analytical experimental method and foresight. Ehrlich's thesis was organized into two parts. In the first part, he overviews the chemical bases of many important histological reactions and, in the second part, he discusses the chemical, technological and histological properties of aniline dyes.

In the chapter dedicated to the histological applications of this class of chemical compounds, he presents his personal point of view about a type of cell, which he named as 'mast cell' ('Mastzelle'). He stated that 'aniline dyes display an absolutely characteristic behaviour toward the protoplasmic deposits of certain cells' that were 'chemically so sharply' distinguished from the group of Waldeyer's 'Plasmazellen'. With this term, he referred to a broad and heterogeneous category of cells previously described by Waldeyer. Among Waldeyer's 'Plasmazellen', there was a group of connective tissue cells exhibiting large dimension and round shape, which could be distinguished 'from white blood cells on the basis of their significantly large size and lack of contractile activity'. Ehrlich emphasized his assumption that most of those cells that he had described in connective tissues as reactive to aniline staining did not correspond to Waldeyer's description, which was otherwise based on purely morphological criteria, not chemical. 'Anilophilic cells should be strongly separated from ''Plasmazellen''', he insisted. These aniline-reactive cells 'represent *sui generis* elements and must be distinguished from Waldeyer's 'Plasmazellen' by a different denomination'.

He then came to the central part of his presentation. 'From the descriptive point of view', he said, aniline-positive cells should be 'most conveniently described as ''granular cells of the connective tissue'' (''granulierte Bindgewebezellen''); from the physiological standpoint, these cells may provisionally be indicated as mast cells (''Mastzellen'') because, like fat cells, they represent a further development (''Weiterentwicklung'') of the fixed cells of connective tissue'. Ehrlich's concept is absolutely remarkable in that, although mast cells 'are localized with extremely high frequency around blood vessels in the loose connective tissues', 'it seems not justified to regard them as members of a perivascular system'. He also provided a notable explanation to support his view: aniline-reactive cells indeed 'have a tendency to collect around developing preformed structures in connective tissues'. In discussing this point, he added that 'in certain acinar glands (goat parotid), the pattern of mast cell accumulation [inside the organ] is not determined by the branching of the vascular system but by the ramification of the gland excretory ducts'.

In the course of his dissertation, Ehrlich underlined the concept that mast cells must be principally distinguished on the ground of their reactivity to aniline dyes, not simply by their shape and morphological appearance. 'Granular cells are characterized by the presence of a still undetermined chemical substance', 'which is bound to the granular storages in the protoplasm' and which reacts to aniline dyes giving typical metachromasia. The binding of this chemical substance to aniline dyes shows different staining: red–violet with cyanine, orange with fuchsin and red with dahlia and gentian.

Finally, he provided an extremely precise description of mast cell microscopical features. 'The typical aspect of ''granular cells'' is as follows. The mostly stainless protoplasm appears as being filled by more or less numerous grains of variable size. These granules exhibit subtle nuances specific for each staining procedure. The nucleus is mostly not stainable, even in samples which otherwise display beautiful nuclear staining reactions. In flattened cells, the nucleus appears as a characteristically clear spot, due to the absence of the coloured granules and this picture nearly gives the impression of a lacuna in the cell body'.

Many interesting aspects of Ehrlich's dissertation deserve some comments. He first coined the term 'Mastzellen' to describe the aniline-reactive granular cells he found in connective tissues. The German word 'Mast' (from the Greek μαστός = breast) implies a nourishing and 'suckling' function for these cells. Certainly, mast cells do not provide nutrients in a strict sense; however, they are deeply involved in the 'trophism' of tissues. Mast cells are increasingly being recognized as key cells for connective tissue homeostasis, remodelling and repair. They also express relevant angiogenic activity. Their granules indeed contain proteases and cytokines that are known to exert 'trophic' effects (survival, growth and chemotactic) on different cells, such as fibroblasts, myofibroblasts, smooth muscle cells, neurones and endothelial cells. Therefore, the 'provisional' term 'Mastzellen' seems more and more appropriate for describing these cells.

Ehrlich also observed that mast cells did not strictly belong to a diffuse perivascular system (according to Waldeyer's concept of 'Perivaskuläresysteme'), despite their characteristic arrangement close to capillaries. This is indeed an absolutely correct statement. Mast cells often

localize far from blood vessels and also express a series of biological properties that are not related to microvascular functions. He argued that mast cells could also be found around areas of developing tissues. The close relationship between mast cells and tumour growth is of extreme actual interest in the sense proposed by Ehrlich.

In addition, he pointed out that the use of aniline dyes was of the utmost importance for identifying mast cells. Reactivity of aniline with a 'still undetermined chemical substance' stored in the granules was the sole reliable procedure that would enable the microscopist to recognize these cells with certainty. We now know that aniline dyes interact with the highly acidic glycosaminoglycan residues contained within mast cell granules. This reaction, in turn, determines the characteristic metachromasia of such structures. We acknowledge that his advice, not simply to consider cell morphology but to base cell identification upon a specific histochemical reaction, was an extremely modern concept.

As to the origin of mast cells, we now know that they do not differentiate from fibroblasts, as suggested by Ehrlich. He could not imagine, however, that these cells would derive from precursors of the haematopoietic lineage and complete their differentiation in peripheral tissues. This was certainly more than he could determine with the simple support of a light microscope and some histological dyes.

On 17 January 1879, the Physiological Society of Berlin heard a remarkable paper by Paul Ehrlich about the mast cells that he had discovered as a medical student 2 years previously. Ehrlich pointed out that not only do the granules of mammalian mast cells display great avidity for basic dyes, but that they also tend to alter the shade of the dye (metachromasia). Later (with one his pupils), he stressed a second characteristic feature of the mast cell granules in many species, their solubility in water (Westphal, 1891). Michels (1938) wrote that: 'uncounted pages of useless and misleading research have been the result of the failure on the part of many investigators to heed the admonition originally given by Ehrlich and Westphal, that the mast cell granules are soluble in water and that to preserve them tissues must be fixed in 50% alcohol and stained in alcoholic thionine'.

Ehrlich then went on to study the staining reactions of blood cells, laying the foundations of modern haematology on the basis of the specific affinities of the leucocytes for various dyes (Ehrlich, 1891; Ehrlich & Lazarus, 1898). He encountered cells with basophilic, metachromatic granules, and thus came to recognize two types of mast cells: the first – derived from, and living in, the connective tissues (tissue mast cells); the second – the counterpart of the neutrophil polymorph and eosinophil leucocyte – with its origin in the bone marrow and habitat in the peripheral blood (blood mast cell, basophil or mast leucocyte). Meanwhile, Ehrlich (1891) had discovered basophilic granular cells in human blood, although so far only in myeloid leukaemia. Nevertheless, with characteristic insight, he at once perceived that, in higher vertebrates, the blood mast cells are true leucocytes stemming from precursors in the bone marrow. By the time that his textbook (Ehrlich & Lazarus, 1898) was revised in 1909, the evidence for the myeloid origin of the blood mast cell was complete (Jolly, 1900). Later work established that mast cells and basophils share several notable features besides staining properties. Both cell types represent a major source of histamine and other potent chemical mediators implicated in a wide variety of inflammatory and immunological processes.

To study the presence and significance of mast cells in pathological conditions again acknowledges our debt to the pioneer observations of Ehrlich who described two situations in which connective tissue may be overnourished, in chronic inflammation and the environs of tumours. Here, there exists a lymph stasis, a damming up of tissue fluid rich in nutriment, whereby certain fixed connective tissue cells are stimulated to become mobile, to multiply and to convert some of the abundant extracellular material into specific intracellular granules. According to Ehrlich, mast cells were 'indices of the nutritional state of the connective tissue', increasing during periods of hypernutrition, diminishing during periods of relative starvation. Ehrlich found many mast cells in tumours, especially carcinoma, but it was left to his pupil to recognize that the cells tend to accumulate at the periphery of carcinomatous nodules rather than within the substance of the tumour (Westphal, 1891). The number of mast cells within the perivascular and interstitial connective tissue of different neoplasias has been reported to be increased. In some cases, this phenomenon is a characteristic feature of the lesion.

During the 60 years that followed Ehrlich's discovery, the research on the mast cell was almost entirely histological. Controversies arose but their resolution for the most part merely emphasized the soundness of Ehrlich's original work. However, the functional biology of mast cells resisted clarification until recently, as their role in promoting the non-specific inflammatory reaction and in different immune responses could be elucidated. Also, neoplasias arising from mast cells have been elusive to clinicians, haematologists and pathologists.

The origin of mast cells remained obscure for many years. It is now accepted that mast cells arise from pluripotential haematopoietic cells in the bone marrow that express CD34, *c-kit* and CD13 (Kirshenbaum *et al*, 1999). This was demonstrated for the first time by Kitamura *et al* (1978), who performed *in vivo* experiments using genetically mast cell-deficient mutant mice. However, in contrast to other cells of the haematopoietic stem cell lineage, which differentiate in the bone marrow before being released into the circulation, mast cells do not circulate as mature cells, but in small numbers as committed progenitors. The progenitors complete their maturation with concomitant phenotypical diversity after moving into diverse peripheral tissues.

The concept of 'mast cell heterogeneity' has represented a focal point in recent discussions of mast cell biology, and it emphasizes that different mast cell populations exhibit significant variation in multiple, potentially important aspects of their phenotype. Mast cells from different species, from different sites in the same species and even from the same organ in one species can vary in their response to stimuli and inhibitors of mediator release. Observations of

histochemical and functional heterogeneity of mast cells, first given a sound basis in the 1960s by Enerbäck (1966, 1986), are now receiving increasing attention (Galli, 1990). Enerbäck reported that, in contrast to mast cells in rat skin, mast cells in the intestinal mucosa were sensitive to routine formalin fixation and could not be identified in standard histological sections. However, after appropriate fixation and sequential staining with Alcian blue and safranin, the mucosal mast cells stained blue in comparison with the connective tissue mast cells, which stained with safranin and were red.

There is no disease, biological condition or animal model yet identified that exhibits an absolute lack of mast cells from which or in which their biological role might be inferred. Mast cells are most commonly regarded as key effectors in the pathogenesis of allergic diseases. However, an exciting development in the study of mast cell biology was the discovery that mast cells can generate or release various cytokines, which indicate a key role played by mast cells also in diverse pathophysiological processes, such as chronic inflammatory processes, wound healing, angiogenesis, fibrosis and tumours.

We wish to conclude this historical note with the remark that all scientists involved in the field of mast cell research should acknowledge their debt to Ehrlich's pioneering observations. By reading his doctoral thesis, in particular, we cannot help admiring the precocious scientific debut of a far-seeing genius who won the Nobel Prize 30 years later.

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