CORRESPONDENCE

MURINE MAST CELLS (NOT NEUTROPHILS) ARE IMPLICATED IN THE BIOLOGY OF LAMININ

To the Editor:

Tweardy et al propose that neutrophils and their precursors produce laminin.1 The evidence presented is based on studies of 32Dc13, a WEHI-3B (interleukin-3 [IL-3]-dependent cell line) that was found to produce the laminin B2 chain protein, to express the relevant messenger RNA, and to stain with antilaminin antibodies after exposure to neuraminidase.1 While it is true that laminin biology has been implicated in neutrophil motility and attachment,3 the claim that neutrophils produce laminin is flawed by incorrect identification of 32Dc13 cells in Fig 4C and D.1 These electron micrographs illustrate typical immature murine mast cells.5 Immature murine mast cells with this morphology were the only cells present in electron microscopic studies of 32Dc23 (Fig 1, Dvorak AM, unpublished data), performed initially for cell identification purposes in 1981. Immature mouse mast cells, whether examined in vivo6 or in vitro7 are identical. They generally display monolobed nuclei, but nuclei may also be lobular, irregularly shaped, or binucleate, and they contain large immature granules characterized by variable vesicular, membranous, amorphous, and dense materials (Fig 1 [and Fig 4C and D in ref 1]). Smaller dense progranules are located in Golgi areas, in the cytoplasm near immature granules, and inside immature granules. Murine neutrophils and their immature precursors do not display these features.25

The findings of Tweardy et al regarding 32Dc13 cells are of considerable interest, because they extend current studies that implicate mast cells in the biology of laminin.2,26 For example, it has been shown that laminin promotes phorbol myristate acetate (PMA)-activated mast cell attachment,26 a function shown to be associated with an amino acid sequence in the laminin A chain,25,26 and one that is enhanced with IgE-mediated activation.26-27 Several laminin-binding proteins cause the adherence of mast cells to laminin, and the transcript for one of these is present in cultured mouse IL-3-dependent mast cells.24,25 In addition to IgE- (or PMA-) mediated promotion of mast cell attachment to laminin,24,25,27 similar enhancement of attachment occurs when mast cells are stimulated with the calcium ionophore A23187.27 and the

Fig 1. Electron micrograph of 32Dc23, an IL-3–dependent cell line derived from long-term bone marrow cultures of C3H/HeJ mice,7 shows classic ultrastructural morphology of immature mouse mast cells.9-22 The single nucleus is irregular in shape and shows partially dispersed chromatin. Surface processes are narrow, short folds. Immature granules (open arrowheads) are large structures that contain a mixture of dense progranules, myelin membranes, and variably sized vesicles. Free ribosomes and mitochondria are present in the cytoplasm. The Golgi area (G) is expanded; a centriole is nearby. Smooth empty vesicles are budding from Golgi structures and are clustered in perigranular areas. One contains a dense progranule (closed arrowhead). Original magnification ×12,000.
cytokine, transforming growth factor-β, upregulated the IgE-mediated attachment of mast cells to laminin. More recently, mast cells have been shown to synthesize laminin. It should be noted that all of these studies of mast cells and laminin have been accomplished using cultured immature mouse mast cells, dependent on the IL-3 present in WEHI-conditioned media (some have been done with mature mast cells, as well) that are identical by morphologic criteria to the 32Dc13 cells illustrated in Fig 4C and D in reference 1 and to the 32Dc23 cell illustrated in Fig 1.

REFERENCES


14. Dvorak AM: Morphologic expressions of maturation and function can affect the ability to identify mast cells and basophils in man, guinea pig, and mouse, in Befus AD, Bienenstock J, Denburg JA (eds): Mast Cell Differentiation and Heterogeneity. New York, Raven, 1986, p 95


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RESPONSE

To the Editor:

Dr Dvorak makes the interesting point in her letter that 32D cell line clones, clone 23 in her studies and clone 3 in our study, have electron microscopic features of mast cell precursors. This point is not surprising because Greenberger et al demonstrated that the parental 32D cell line produces basophilic/mast cell colonies in vitro when cultured in the presence of interleukin-3 (IL-3). Taken together with the data in our paper, this observation does implicate laminin B2 chain production in the biology of mast cell precursors. However, this conclusion does not in any way detract from our observations demonstrating endogenous laminin B2 chain production in 32D clone 3 (32Dc13) cells undergoing differentiation into mature neutrophils.2

In her letter, Dr Dvorak has ignored the demonstration by us and others that the specific clone of 32D used in our studies, clone 3, is multipotential.3 Specifically, we have clearly demonstrated that when cultured in the presence of granulocyte colony-stimulating factor (G-CSF) the majority, if not all, of 32Dc13 cells differentiated into mature neutrophils.3 The program for neutrophilic differentiation began soon after G-CSF exposure: mRNA levels for myeloperoxidase, a specific marker for neutrophilic granulocytes, appeared within 24 hours of exposure and peaked at 48 hours.4 We demonstrated in our laminin study that after 48 hours of cultivation in G-CSF, 32Dc13 cells continued to express undiminished levels of laminin B2 chain mRNA. Thus, 32Dc13 cells that are committed to becoming neutrophilic granulocytes continue to express mRNA for B2 chain laminin. Also, not commented on by Dr Dvorak in her letter is our finding of surface laminin on mature peripheral blood neutrophils by flow cytometry.5

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REFERENCES

Murine mast cells (not neutrophils) are implicated in the biology of laminin [letter; comment]

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