Conditioned Media Obtained from a Human Mastocytosis Cell Strain Induce Mast Cells Expressing Chymase but not Tryptase from Human Progenitors

L.	Lixin	Li
S.A.	Steven A.	Krilis

School of Medicine, University of New South Wales and Department of Immunology, Allergy and Infectious Disease, St. George Hospital, Kogarah, Australia

Key Words

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c-kit ligand

Abstract

Human mast cells (MC) were derived from umbilical cord blood and bone marrow progenitors cultured in the presence of a conditioned medium from a human mastocytosis cell strain and recombinant human kit ligand (rhKL). KL induced MC of predominantly two immunophenotypes, MC_T and MC_{TC}. In contrast, the conditioned medium induced MC subtypes MC_{TC} and a third subtype, MC_C, positive for chymase but negative for tryptase. This study clearly demonstrates that a third type of MC, MC_C, can be induced in vitro from normal human progenitors.

Correspondence to: Prof. S.A. Krilis, Department of Immunology, Allergy and Infectious Disease, St. George Hospital, Kogarah, New South Wales, 2217 (Australia), Tel. 61 2 93502955, Fax 61 2 93503981, **E-Mail** s.krilis@unsw.edu.au

Introduction

Distinct subclasses of human mast cells (MC) have been described [1]. The MCT expresses one or more tryptases, whereas the MCTC expresses tryptase, chymase, cathepsin G and carboxypeptidase A. Recently, studies using immunostaining with antibodies to tryptase and chymase in tissue MC have indeed suggested that a tryptase-negative MC might exist in humans [2]. Recombinant human kit ligand (rhKL) induces MC in vitro from a variety of sources of human hematopoietic progenitor cells, but the MC are immature [3]. Our group has recently demonstrated that conditioned medium derived from a human mastocytosis cell line (HBM-M) can also induce normal bone marrow progenitor cells to become morphologically mature cells that express FcεRI and the MC-specific proteases tryptase, chymase and carboxypeptidase [4]. In this study, MC subtypes derived from human bone marrow and umbilical cord blood were assessed by a systematic sequential double immunoenzymatic analysis.

Materials and Methods

HBM-M-conditioned medium (HBM-M-CM) was prepared and cultures of human bone marrow and umbilical cord blood cells were performed as described previously [4]. Mouse antitryptase monoclonal and rabbit anti-chymase polyclonal antibodies were used for double immunostaining. Briefly, cytopun cells were incubated with antitryptase antibody after fixation in Carnoy's fixative followed by rabbit antimouse IgG and APAAP complex. Slides were developed in naph-thol AS-MX phosphate solution containing fast red. Subsequently, cells were incubated with antichymase antibody (kindly provided by Dr. N. Schechter, USA) followed by

sheep antirabbit-HRP. The slides were then treated with a freshly prepared 3,3-diaminobenzidine tetra-hydrochloride solution. In these immunohistochemical reactions, MCT are stained red, whereas MCC are stained yellow-brown. MC-reappear as both colors when stained.

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Results and Discussion

Bone marrow cells cultured with HBM-M-CM contained predominantly MCTC and MCT subtypes. The percentage of MCC in total MC decreased overtime from 100% at day 10 to 56.5% at day 21, when the percentage of MCTC was maximal and represented 37.4% of total MC in the culture. When human MC were derived by culturing umbilical cord blood cells in the presence of rhKL alone, the MCT cells were the major MC population at the early time points. The numbers of MCTC were increased slightly after 4 weeks of culture. After 5 weeks, 63, 30 and 7% (n = 5) of the total MC in the umbilical cord blood cultures were of the MCT, MCTC and MCC subtypes, respectively. In contrast, cultures supplemented with both HBM-M-CM and rhKL resulted in 57.9% MCTC at day 49 and 61.3% MCC at day 21. However, the number of MCT cells on day 49 was only 26% (n = 6) (fig. 1). Using the conditioned media and cytokine combination, we derived a novel population of human MC that does not express tryptase. The HBM-M-CM induced MCC and MCTC cells with downregulation of the MCC cells and the appearance of MCT cells when rhKL was added to the cultures. Based on ultrastructure and differences in protease composition between the MCT and MCTC types of MC, it has been postulated that there are distinct developmental pathways for these cells. The fact that the expression of MCC and MCT can be reversibly altered by culturing these MC in the presence and absence of HBM-M-CM indicates that in the human the phenotype of MC with respect to protease expression is not fixed. This has been elegantly demonstrated in vitro with bone-marrow-derived rodent MC cultured in the presence of different combinations of cytokines [5]. The results of this study would suggest that hu-

Fig.1. The percentage of MC subtypes of total MC in umbilical cord blood cultures with HBM-M-CM and KL at different time points.

man MC in culture are in a dynamic state and their protease phenotype is regulated by cytokines in the microenvironment.

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