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Rapid Communication

Cell membrane associated free kappa light chains are found on a subset of tonsil and *in vitro*-derived plasmablasts

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ABSTRACT

The monoclonal antibody, MDX-1097, is currently progressing through clinical trials as a possible therapy for multiple myeloma. MDX-1097 targets a cell membrane bound form of free immunoglobulin kappa light chain (FκLC), termed kappa myeloma antigen (KMA), which is found on the surface of malignant plasma cells. The clinical potential of MDX-1097 highlights the need to characterise the expression of its cognate antigen, KMA, in normal tissue. In this study, we have analysed the expression of KMA on B cell subsets found in tonsils, peripheral blood and bone marrow. We found KMA expression on a small population of tonsillar and *in vitro* derived plasmablasts. In contrast, no KMA expression was observed on peripheral blood or bone marrow resident B cell subsets. This study yields important insights into the possible subsets of B cells that might be depleted as a result of an immunotherapy targeting KMA.

1. Introduction

Kappa myeloma antigen (KMA) is a plasma membrane associated form of free immunoglobulin (Ig) kappa light chain (FκLC) expressed on malignant plasma cells from patients with kappa type multiple myeloma (κMM), Waldenström's macroglobulinaema and non-Hodgkin's lymphoma [1–3]. KMA is recognised by the murine mAb, K-1-21, and its human-mouse chimeric equivalent, MDX-1097 [1,4], which was recently assessed in a Phase IIa clinical trial as a therapy for κMM (ANZCTR: #12610000700033). While the initial analysis of normal cells and tissues with K-1-21 failed to detect the expression of KMA on antibody secreting cells and resting B cells from healthy adults, the antigen was detected on some foetal B cells and a small proportion of *in vitro* activated B cells [1,2]. Thus, the development of a chimeric version of the antibody for potential therapeutic use warranted a re-examination of the expression of KMA on normal B cell subtypes.

In this study, we have characterised KMA expression on B cells from a variety of healthy human tissues, and found that it is limited to a subpopulation of plasmablasts residing in tonsils. Interestingly, KMA expression is entirely absent from peripheral blood and bone marrow B cell subsets. Since the KMA-specific antibody, MDX-1097, is being assessed clinically as a therapeutic for κMM, our study provides important insights into the possible subsets of B cells that might be depleted by an immunotherapy targeting KMA.

2. Materials and methods

2.1. Staining reagents and flow cytometry

The following mAb conjugates were used in this study: anti-CD27 FITC/PE (clone M-T271) and anti-CD38 PE-Cy7 (clone HIT2; BD Biosciences); anti-CD19 PE (SJ25-C1; Sigma). The anti-FκLC mAb, K-1-21, was affinity purified from hybridoma culture supernatant. K-1-21 and MOPC21 mouse IgG1 isotype control (Sigma) were then labelled with allophycocyanin via sulfhydryl conjugation.

Abbreviations: FκLC, free immunoglobulin kappa light chain; Ig, immunoglobulin; κMM, kappa type multiple myeloma; KMA, kappa myeloma antigen; MNC, mononuclear cells.

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Cells were stained with a cocktail of mAbs and SYTOX Blue (Life Technologies) to exclude dead cells. Flow cytometry was performed on an LSR II and FCSExpress (De Novo) was used to analyse results.

2.2. In vitro differentiation of CD19$^+$ peripheral blood B cells

Buffy coats from healthy blood donors were obtained from the Australian Red Cross Blood Service under ethics approval. CD19$^+$ cells were positively selected from Ficoll purified mononuclear (MNC) cells using CD19 specific magnetic beads (Miltenyi Biotec). B cells were then suspended at 5×10^5 cells/mL in RPMI-1640 with 10% foetal bovine serum supplemented with 100 units/mL penicillin and 100 μg/mL streptomycin. Following a method adapted from Ettinger et al. [5], samples were incubated for 6 days with a cocktail of 100 ng/mL IL-21 (R&D Systems), 1 μg/mL anti-CD40 (R&D Systems) and 5 μg/mL goat polyclonal anti-IgM (Sigma).

2.3. Sources of MNCs

Human tonsils were obtained from routine tonsillectomy at the Royal Prince Alfred Hospital (Sydney, Australia) and St Vincent's Hospital (Darlinghurst, NSW). All studies were approved by institutional Human Research Ethics Committees. Bone marrow MNCs were sourced from Stem Cell Technologies, Inc. Cells were cryopreserved in liquid nitrogen before use.

3. Results

3.1. KMA is expressed on in vitro-derived plasmablasts

An earlier study had shown that KMA expression could be induced on tonsil-derived B cells through *in vitro* activation with formalin fixed *Staphylococcus aureus* [2]. It was presumed that these KMA$^+$ cells had differentiated into FκLC-secreting plasmablasts, however they were not phenotyped at the time [2]. As an initial experiment to characterise KMA expression, we utilised an *in vitro* B cell differentiation system to assess KMA expression on B cells in various states of activation/differentiation. Peripheral blood B cells were purified by CD19$^+$ selection using magnetic beads and, prior to *in vitro* stimulation, were analysed by flow cytometry to characterise the expression profile of KMA on these cells. In agreement with earlier studies, no KMA expression was observed on peripheral blood B cells (Fig. 1 and Table 1) [1,2].

Peripheral blood B cells were then stimulated with a cocktail of IL-21, anti-CD40 and anti-IgM, which can differentiate large

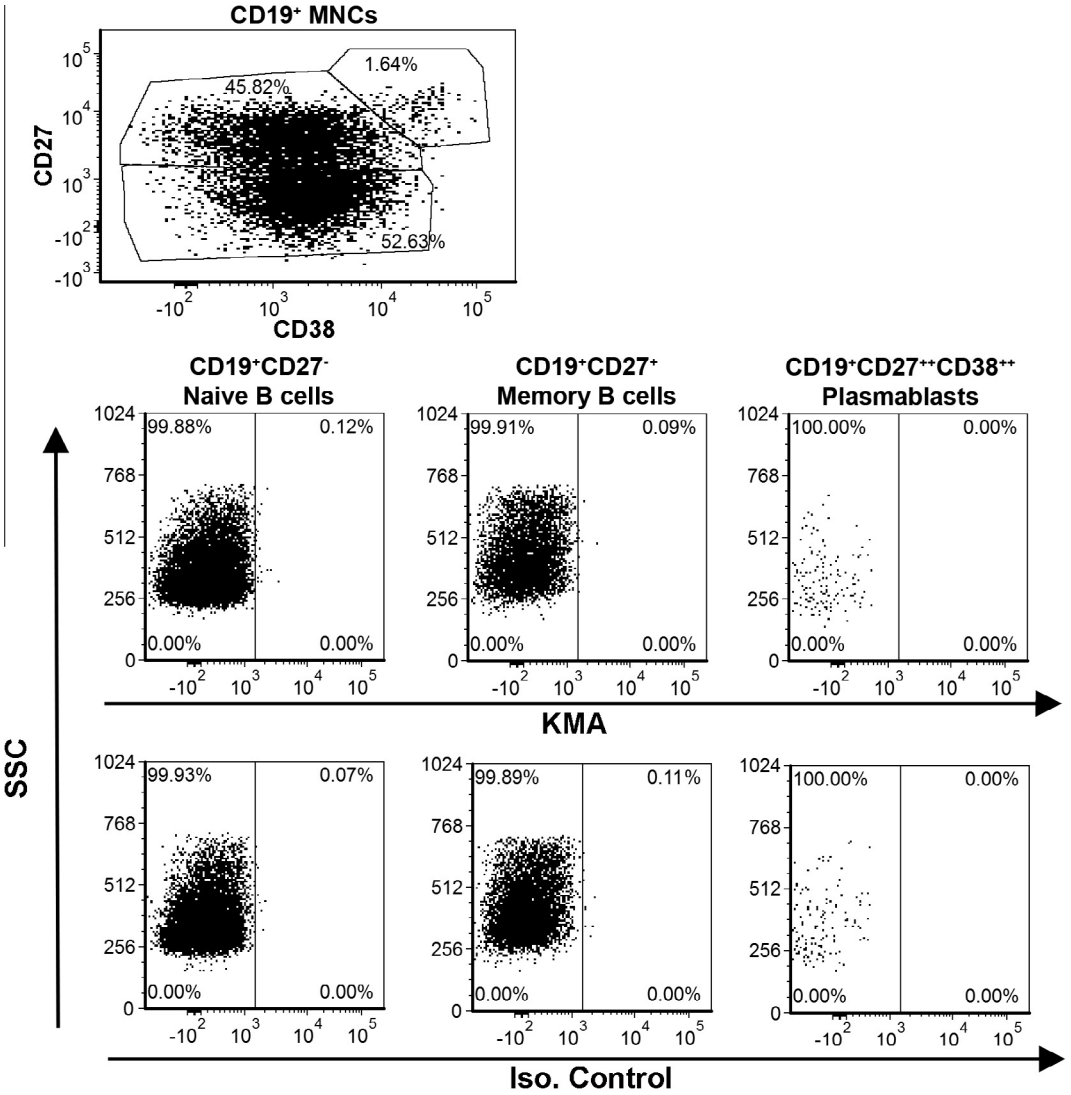

Fig. 1. Lack of KMA expression on peripheral blood B cell subsets. CD19$^+$ B cell subsets were gated according to the expression of phenotypic markers and then assessed for KMA expression.