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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

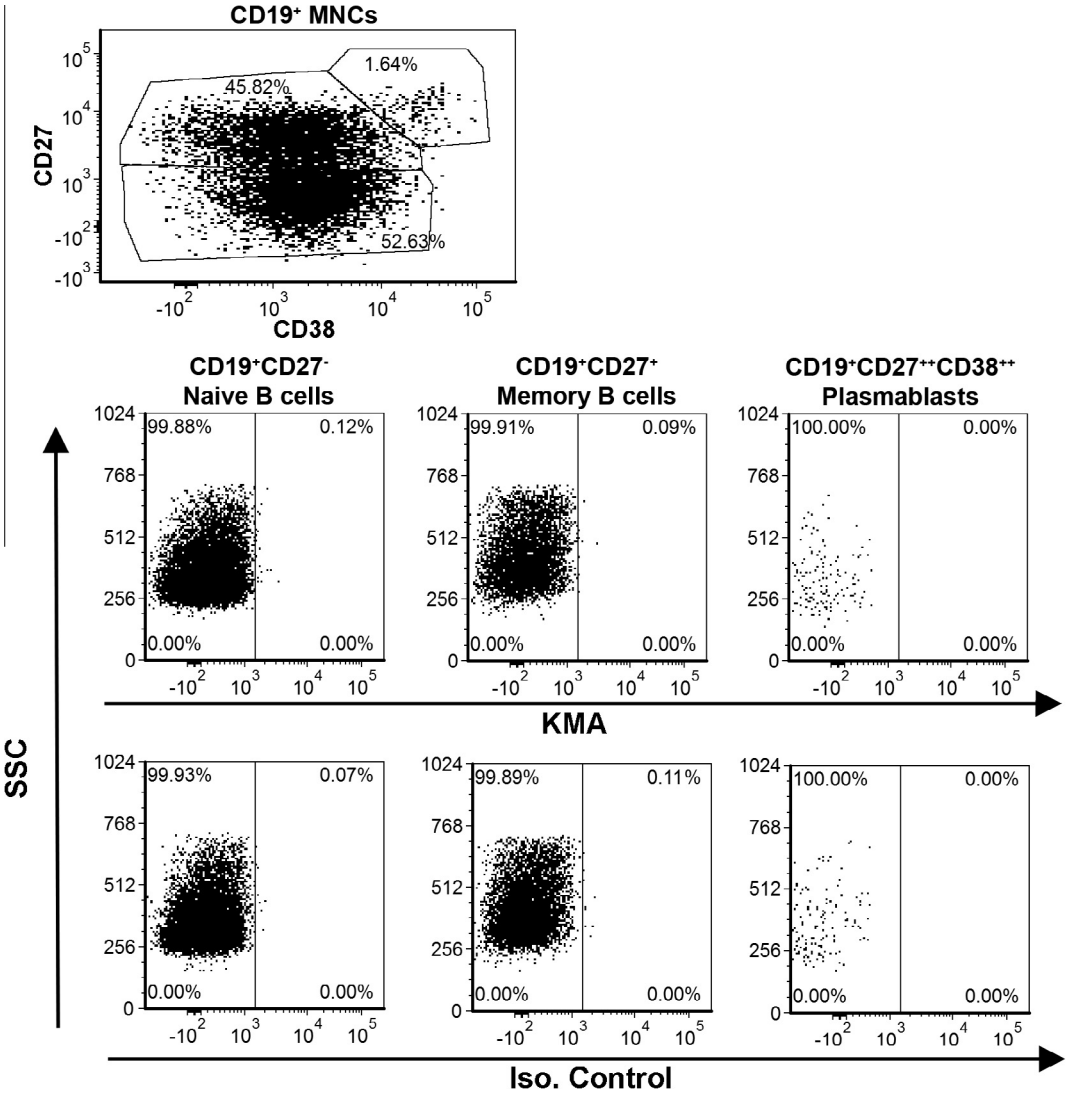

Fig. 1. Lack of KMA expression on peripheral blood B cell subsets. CD19$^+$ B cell subsets were gated according to the expression of phenotypic markers and then assessed for KMA expression.

Table 1
Expression of KMA on $CD19^+CD27^{++}CD38^{++}$ MNCs sourced from various tissues.

Source	*n*	% KMA^+ cells within $CD19^+CD27^{++}CD38^{++}$ compartment		
		Mean	Range	SD
In vitro differentiated B cells	6	9.7	2.7–12.4	3.6
Tonsil	4	4.6	1.8–11.1	4.4
Peripheral blood	5	0	0	0
Bone marrow[a]	3	0	0	0

[a] *Note:* bone marrow samples were gated according to $CD19^{+/-}CD38^{++}$ only. KMA was entirely absent on these cells so no further analysis was performed.

numbers of B cells into $CD27^{++}CD38^{++}$ plasmablasts [5,6]. Six days after stimulation, cells were harvested and then phenotypically assessed by flow cytometry. Success of the treatment was indicated by the high proportion of cells that had differentiated into plasmablasts as defined by high expression of CD38 and CD27 [7,8] (mean proportion of cells with a plasmablast phenotype was 73.6%; Fig. 2A). Further analysis revealed that a small population of these cells expressed KMA (12.08% KMA^+ cells above the isotype control in the representative plot; Fig. 2). This was consistent across all six donors with each sample showing some level of KMA expression within the $CD27^{++}CD38^{++}$ plasmablast population of cells (Table 1).

3.2. KMA is found on a subset of human tonsillar plasmablasts

Since KMA was expressed by a small subset of *in vitro*-derived plasmablasts, we set out to determine whether similar subsets could be found in B cell-rich tissues such as human tonsils and bone marrow. Analysis of KMA expression on specific B cell subsets revealed a small population of KMA expressing $CD19^+CD27^{++}CD38^{++}$ plasmablasts in tonsillar tissue (5.40% of cells above the isotype control; Fig. 3). This was consistent across all donors with mean KMA expression on tonsil-resident plasmablasts of 4.6% (Table 1). In contrast, there was little to no KMA expression within the other populations of B cells (Fig. 3). Analysis of MNCs derived from bone marrow revealed that KMA was not expressed by $CD19^+$ B cells or $CD19^{+/-}CD38^{++}$ Ig-secreting cells (Table 1 and Supplementary Fig. 1). This was similar to our findings for peripheral blood where no KMA expression was found on these cell types (Fig. 1 and Table 1). Together these results suggest that KMA is only expressed by a small subpopulation of plasmablasts within tonsils, and is absent from B cell subsets residing in the peripheral blood and bone marrow of normal adults.

4. Discussion

The anti-KMA chimeric mAb, MDX-1097, is currently being tested in the clinic as a potential therapy for κMM [9]. An early study utilising the murine mAb equivalent of MDX-1097, termed K-1-21, revealed that expression of KMA was primarily limited to malignant Ig-secreting cells with no KMA^+ cells found in normal adult tissues. This same study, however, showed that KMA expression could be induced in normal tonsil-derived B cells after *in vitro* activation. These *in vitro*-induced KMA^+ B cells were presumed to correspond to Ig-secreting cells, however no further phenotypic characterisation was performed [2]. In this current study we report, for the first time, expression of KMA on a subset of *in vitro*-derived and tonsillar $CD27^{++}CD38^{++}$ plasmablasts. While we cannot conclusively rule out the possibility that some of these KMA^+ cells are plasma cells, all KMA^+ cells co-expressed CD45 (data not shown), which can be used as a marker to delineate between cycling immature Ig-secreting cells (i.e. plasmablasts) and terminally differentiated plasma cells [10]. Furthermore, the fact that KMA expression is found on Ig-secreting cells in tonsils and not in more mature compartments such as the peripheral blood or bone marrow further supports the conjecture that these

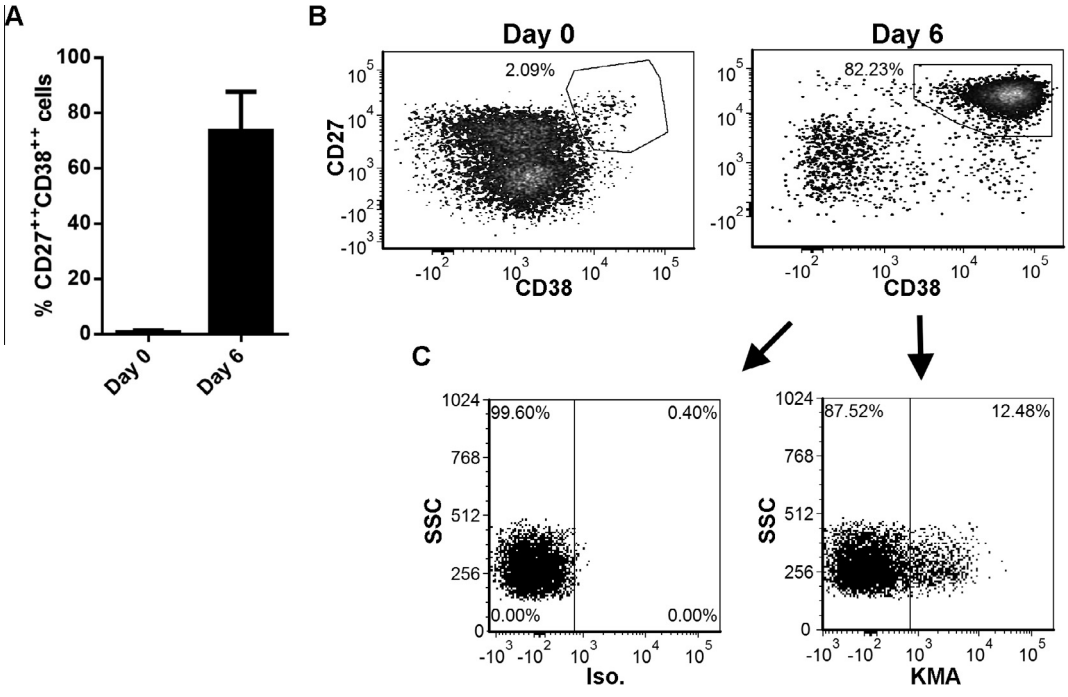

Fig. 2. Expression of KMA on *in vitro* derived plasmablasts. Peripheral blood $CD19^+$ B cells were differentiated by incubation with IL-21, anti-CD40 and anti-IgM. Cells were then analysed by flow cytometry for the expression of various phenotypic markers and KMA. (A) The percentage of $CD27^{++}CD38^{++}$ plasmablasts within the total viable cell population at Day 0 and Day 6 respectively ($n = 6$ donors). (B) Representative dot plot showing expression of CD27 and CD38 on peripheral blood B cells at 0 and 6 days differentiation. (C) Representative dot plots showing the levels of KMA on 6 day differentiated $CD27^{++}CD38^{++}$ plasmablasts.

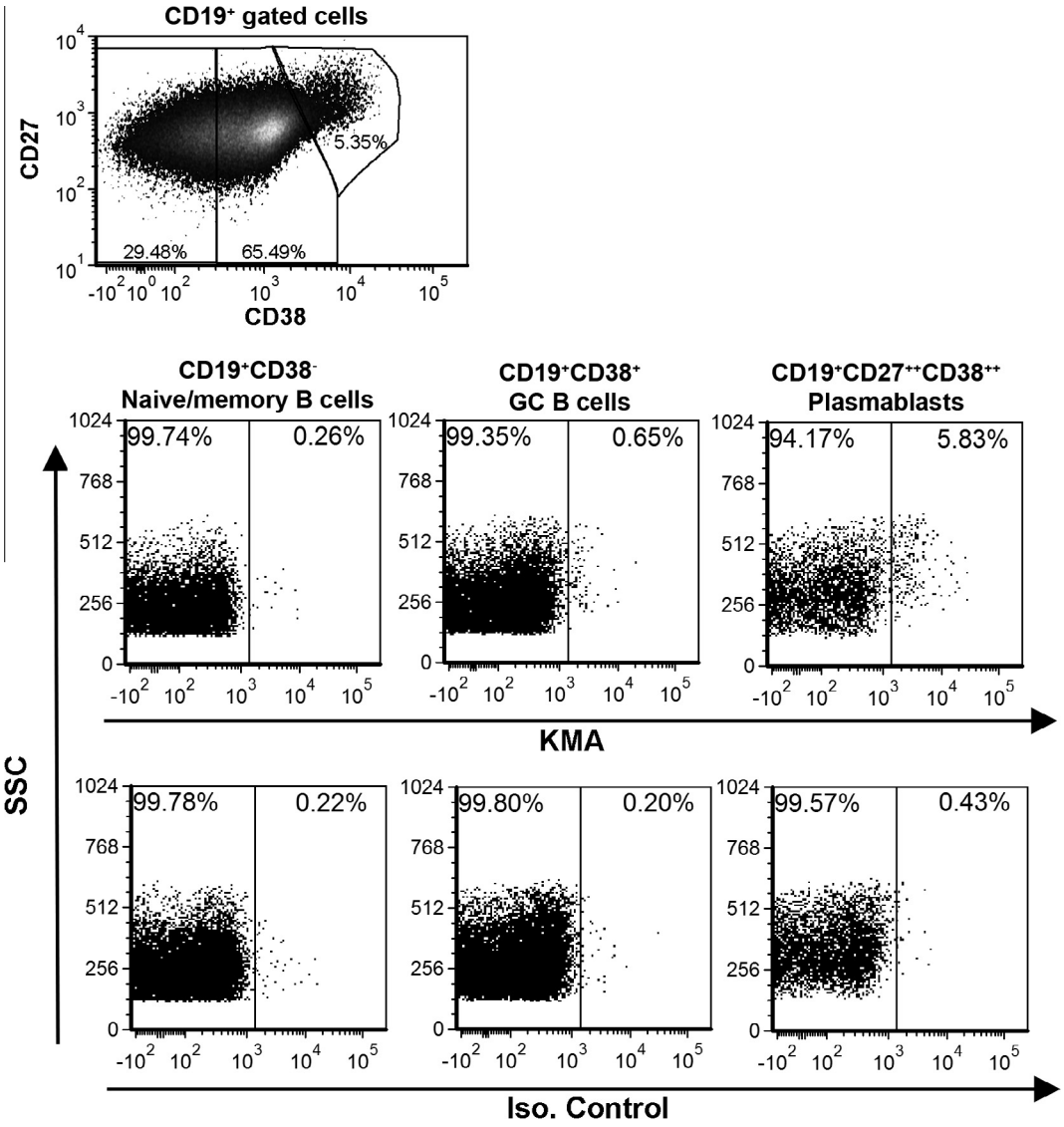

Fig. 3. A subset of CD27^{++}CD38^{++} tonsillar plasmablasts express KMA. Tonsil mononuclear cells were stained for KMA with various phenotypic markers, and then assessed by flow cytometry.

cells are plasmablasts. It is also worth noting that our study did not look at other lymphoid tissues (e.g. lymph nodes, spleen and mucosa-associated lymphoid tissues). Thus it is possible that KMA expressing B cells may also be found in other tissues, especially those that are sites of B cell differentiation.

We have shown that KMA is a cell membrane-associated form of FκLC [3,11,12]. The KMA complex consists of FκLC bound to saturated zwitterionic phospholipids. Furthermore, cell membrane-associated FκLCs, as KMA, reside in an aggregated form which is thought to stabilize the complex to the lipid bilayer (i.e. through a low affinity but high avidity interaction). The membrane association of aggregated FκLC presumably occurs within secretion vesicles, and after exocytosis, the complex becomes exposed on the extracellular face of the cell membrane as KMA [3,12]. The mechanism of FκLC aggregation and thus expression of KMA is not well understood. For example, some FκLC expressing MM cells do not express KMA [3], and in the current study, expression of KMA was only found on a small subset of normal plasmablasts. One possibility is that KMA^{+} cells express highly aggregation prone FκLCs as a result of hypermutation events during B cell development and differentiation. Another possibility is that KMA expression by plasmablasts and MM cells is similar to what has previously been described for cell membrane-associated insulin on islet cells. This is thought to arise from the secretion of insulin containing dense-core granules which slowly dissipate from the cell membrane and can be detected by immunofluorescence [13]. Although a characteristic of most secretory cells is the presence of dense-core granules [14,15], normal Ig-secreting cells do not possess these in high abundance [16]. Thus further studies will be required to determine if there is a relationship between the presence of dense core secretory granules and KMA expression.

A number of mAb therapies are currently being assessed as possible treatments for MM [17]. Of the mAbs that target tumour cells directly, all are raised against antigens that are also expressed by other cell types. For example, elotuzumab (BMS, Abbott) recognises CS-1/CRACC (CD319) which is expressed on a variety of cells including plasma cells, NK cells, CD8^{+} T cells and dendritic cells [18–20], and daratumumab (Genmab, Janssen Biotech Inc.) is specific for CD38, which is also found on a wide lineage of leukocyte populations [21]. This potential binding of therapeutic mAbs to non-malignant cell types raises concerns of off-target effects, and therefore any truly specific MM cell marker would be highly valued

as a therapeutic target. For this reason KMA was considered a safe and effective antigen for κMM. While this study reveals that the expression of KMA is not solely restricted to malignant plasma cells, our results nevertheless show that KMA expression is limited to a minor population of normal cells, and for this reason an anti-KMA mAb is unlikely to have significant off-target effects when administered therapeutically. This assessment is supported by the clinical success of the anti-CD20 mAb, rituximab, in the treatment of non-Hodgkin's lymphoma [22]. While rituximab targets all $CD20^+$ B cells, the consequent depletion of the normal B cell population is readily managed at the clinical level [23]. The validity of KMA as a target for immunotherapy is further supported by the results of Phase I and IIa clinical trials with the chimeric anti-KMA mAb, MDX-1097, which showed that the mAb was well tolerated with no dose limiting toxicity [9].

Disclosures

RLR is a shareholder of Immune System Therapeutics Ltd. ATH, DRJ & PMW are former employees of Immune System Therapeutics Ltd.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.humimm.2014.08.196.

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