Expression of the Kappa Myeloma Antigen (KMA) on the Cell Surface of Bone Marrow Aspirates from Waldenstrom's Macroglobulinemia Patients

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Introduction

Waldenstrom's Macroglobulinemia (WM) is an uncommon B cell neoplasm characterised by lymphoplasmacytic lymphoma and IgM monoclonal gammopathy. WM is defined as a distinct clinicopathologic entity on the basis of the presence of pleomorphic B-lineage cells, such as small lymphocytes, lymphoplasmacytoid cells and plasma cells in the bone marrow and lymph nodes. The bone marrow (BM) is infiltrated predominantly in an intratrabecular pattern and there is evidence for the presence of significantly increased numbers of mast cells which are suggested to play a supporting role in the growth of plasmacytic cells (Tournilhac et al., 2006). Although variations in the immunophenotypic profile of WM exist, the typical phenotype consists of pan-B-cells markers CD19, 20 and 22, combined with cytoplasmic Ig, FMC7, BCL2, PAX5, CD38 and the absence of CD10 and CD23 (Vijay and Gertz, 2007) with the most common presenting phenotype being surface IgM (sIgM), CD19, CD20 positive and CD5, CD10 and CD23 negative (Owen et al., 2003). Additionally, it has been shown that CD27 is heterogeneously expressed in WM cells (San Miguel et al., 2003, Kriangkum et al., 2004).

We have studied archived BM cells from diagnosed WM patients using Immune System Therapeutics' (formerly PacMab) mAb: mKap, which in addition to recognising soluble kappa Light chain (κLC), is capable of binding Kappa Myeloma Antigen (KMA; Walker et al., 1985). KMA is a membrane associated form of κLC, present on the cell surface independent of the Ig heavy chain. We have previously shown that KMA is present on the surface of a proportion of BM aspirate cells from κ-type Multiple Myeloma (MMκ) patients and on MMκ cell lines. In this study we used flow cytometry to demonstrate the presence of KMA on the surface of a subpopulation (10-15%) of sIgM+ cells from 3 of the 7 WM patients expressing κ isotype. Furthermore BM cells from 2 of 3 patients contained a subpopulation which was KMA+ and sIgM negative. In addition to sIgM and KMA status the cells were analysed for expression of CD27 and CD38. It was shown that both sIgM+/KMA+ and sIgM-/KMA+ cells expressed CD38 and CD27.

Interestingly, we have been able to demonstrate that when peripheral blood derived B cells are subjected to an *in vitro* process of activation and differentiation (using IL21, anti-IgM and anti-CD40 antibodies) they express KMA and that this expression is concurrent with the expression of CD27 and CD38. These findings may provide insights into the etiology of immunophenotypically divergent neoplasms such as WM and MM.

Materials and Methods

WM BM samples

Archived WM BM aspirates were obtained from Peter MacCallum Cancer Centre Tissue Bank, Melbourne, Australia. Vials were thawed rapidly in 37° C waterbath, layered with 5mL FCS and centrifuged at 400g for 5 minutes. The cell pellet was washed in 10mL FACS staining wash (FSW; PBS, 1% BSA, 0.02% sodium azide) before counting and FACS staining.

Antibodies

Directly conjugated, commercially available antibodies to CD27, CD38, IgM and IgD were obtained from BD Biosciences. KMA was detected using biotinylated mKap. The monoclonal antibody mKap was produced by IST's hybridoma cell line under GLP conditions. Purified antibody was biotinylated using the EZ-Link Maleimide PEO solid phase biotinylation kit (Pierce, USA). A MOPC control antibody was also labeled in a similar fashion (b-MOPC). The resulting labeled mKap (b-mKap) was characterized with regards to its KMA binding specificity and sensitivity using the myeloma cell line JJN3.

FACS staining and analysis

Generally, cells were incubated for 30 min on ice with b-mKap (or b-MOPC). After washing in FSW, the directly conjugated antibodies and streptavidin-APC (Molecular Probes) (used for detection of b-mKap) were added. The cells were then incubated a further 20 min on ice in the dark, washed and analyzed on a FACSCalibur flow cytometer.

in vitro B cell activation and differentiation

Peripheral B cell activation was performed following the method described by Ettinger et al. (2005). Briefly, human peripheral B cells were isolated by positive selection via anti-CD19 microbeads (Miltenyi Biotec) from buffy coats of anonymous and consented donors obtained from the Australian Red Cross. Preparations were typically > 95% pure. Purified B cells were cultured in 24 well flat bottomed plates at 1 x 10^6 cells/mL in RPMI/10%FBS with human IL-21 (100ng/mL; R&D systems), anti-human CD40 (1μg/mL; R&D Systems) and anti-human IgM (5μg/mL; Sigma Aldrich). After 6 days in culture, cells were harvested and stained as above.

Results

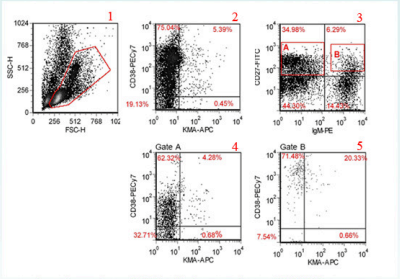

Figure 1. KMA is expressed on the surface of BM cells from patients with WMκ

Panel 1: The scatter profile of BM derived cells is shown. Gated population selected for analysis excludes dead cells as indicated by uptake of propidium iodide (data not shown).
Panel 2: Approximately 80% of cells are CD38+. The signal spans both intermediate and bright levels. Approximately 5.4% of cells are CD38+/KMA+.
Panel 3: Approximately ~35% of cells are CD27+/IgM- (Gate A) while 6.3% are CD27+/IgM (GateB).
Panel 4: Further analysis of Gate A, Panel 3 (CD27+, IgM-) shows that 4.3% of cells are KMA+ with intermediate CD38 staining intensity.
Panel 5: Further analysis of Gate B, Panel 3 (CD27+, IgM+) shows that 20% of cells are KMA+ with bright CD38 staining intensity.

Patient		IgM+, CD27+, CD38+ mKap (%)	IgM+, CD27+, CD38+ iso. contol (%)	IgM-, CD27+, CD38+ mKap (%)	IgM-, CD27+, CD38+ iso. control (%)
κ-type	1	20.3	(2.0)	4.2	(1.0)
	2	30.0	(10.0)	14.2	(7.8)
	3	6.5	(2.0)	5.4*	(3.7)
λ-type	4	2.6	(3.7)	4.4	(2.7)

Table 1. KMA is present on both IgM+ and IgM- subpopulations of WMκ BM cells

The table summarises the results of four WM patient samples (three κ-type and one λ-type as negative control). The cell percentages were calculated for gated populations as discussed in Figure 1. The % KMA+ cells (mKap %) is presented compared to values obtained for a similarly labeled isotype control antibody (iso. control %).
* indicates one patient sample which was KMA- in the IgM- population.

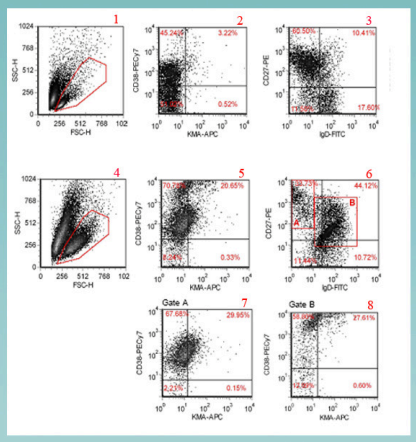

Figure 2. KMA is expressed on *in vitro* activated peripheral blood B cells

Peripheral blood B cells were isolated from buffy coats of normal volunteer donors by positive selection with anti-CD19 microbeads. Cells were cultured in the presence of IL21, anti-CD40 and anti-IgM antibodies for 6 days. Panels 1, 2 and 3 demonstrate the scatter profile and phenotypic status of unstimulated B cells. Generally, the percent cells positive for IgD, CD27 and CD38 remain within normal ranges. There is a slight increase in the percentage of KMA+ cells (1.9% for isotype control compared to 3.22% for mKap).
Panels 4-8 show the results of the analysis of stimulated cells:
Panel 4: The scatter profile of stimulated cells is shown. Gate indicates viable cells which are considered for analysis.
Panel 5: Approximately 30% of cells are CD27+/IgD- (Gate A) while 58% of cells are CD27+/IgD+ (Gate B).
Panel 6: Approximately 71% of cells are CD38+. The CD38 signal spans the intermediate and bright levels. After stimulation approximately 21% of cells are CD38+/KMA+
Panel 7: In the CD38+/IgD+ population (Panel 5, Gate A) approximately 30% of cells are KMA+.
Panel 8: In the CD38++/IgD- population (Panel 5, Gate B) approximately 28% of cells are KMA+.

Summary

Heterogeneity of the malignant phenotype in WM is well documented. In this context we have demonstrated that:

- The novel marker KMA is expressed on both IgM+ and IgM- cells in BM WMκ
- KMA expression is strongly associated with CD27 and CD38 positive cells
- KMA is expressed on a higher proportion of CD38 bright cells than CD38 intermediate cells
- This apparent correlation of KMA with CD38 expression in WMκ cells is consistent with our finding of CD38 and KMA expression on *in vitro* stimulated normal peripheral B cells
- Similarly, CD27 is correlated with KMA expression on *in vitro* normal stimulated peripheral B cells

At present a chimeric version of mKap is in clinical development for a Phase I/IIa clinical trial in MMκ patients. We propose that this antibody would also have therapeutic potential in WMκ patients.

References

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Acknowledgements

Biospecimens were provided by the Peter MacCallum Cancer Centre Tissue Bank, a member of the ABN-Oncology group, which is supported by National Health and Medical Research Funding.

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