Supplemental Materials and Methods

Immunoglobulin gene rearrangement analysis

Total RNA was isolated using the Trizol method (Life Technologies, Carlsbad, CA), quantitated on a NanoDrop 2000 spectrophotometer (Thermo Fisher, Waltham, MA) and 1 µg of RNA was converted to cDNA using the BioRad iScript cDNA kit (Hercules, CA). The cDNA reaction was then diluted to 50 µl with distilled water and 2 µl of cDNA was amplified in each of fourteen separate polymerase chain reactions (PCR). Seven of the PCR reactions used sense V_H family-specific framework region consensus primers for the seven human Ig V_H gene families in conjunction with a specific antisense Cµ primer. Six of the PCR reactions used 5' primers designed to hybridize to the six human Vκ gene families in combination with a single 3′ degenerate primer that hybridizes to the IGK constant region gene. The fourteenth reaction used primers specific for β–actin. The PCR reactions were carried out using a HotStarTaq PCR Kit (Qiagen, Valencia, CA, USA) in a total volume of 50 µl and included 20 pmol of each primer, 200 µmol/l of each dNTP, and 2·5 U HotStarTaq. An Applied Biosystems 9700 Gene Amp thermocycler was used as follows: denaturation at 94°C for 15 min; 34 cycles of 30 s at 94°C; 1 min at 60°C; 1 min at 72°C; and a final cycle of 10 min at 72°C. PCR products were analyzed on 1·5% agarose gels and visualized with ethidium bromide staining. PCR products were purified with a Wizard PCR and Preps kit (Promega, Madison, WI, USA) and sequenced using an automated sequencer (Applied Biosystems, Foster City, CA, USA). Resulting sequences were aligned with germ line sequences in the IMGT/V-Quest reference database.

Immunophenotypic analysis

Cells were incubated with primary monoclonal antibodies for 30 minutes at 4°C. Cells were then washed with Dulbecco phosphate-buffered saline and fixed with 1% paraformaldehyde. Cells were analyzed using a FACSCAN flow cytometer (BD Biosciences Pharmingen, San Diego, CA) and Flowjo analytical software (TreeStar, Ashland, OR). All antibodies were purchased from BD Biosciences Pharmingen except CD49d and CD49e (Serotec, Raleigh, NC). Delta mean fluorescence intensity (MFI) was calculated from the MFI of cells unimodally expressing the marker divided by the MFI of the cells stained with isotype control.

Western blot analysis

Cells (5×10^6) were lysed in lysate buffer (50 mM Tris pH 7.4, 150 mM NaCl, 1% NP-40, 0.5% DOC, 0.1% sodium dodecyl sulfate (SDS), 1 mM EDTA, 15 mM sodium molybdate, 1 mM NaF) and lysates were cleared of insoluble material by centrifugation for 10 min at 14 000 r.p.m. Lysates were then quantitated using Bradford reagent (Bio-Rad, Hercules, CA) and 40 μg of protein lysate were added to an equal volume of 2X SDS loading buffer, heated to 100°C for 5 min, resolved by SDS–PAGE, and transferred to Immobilon-P membranes (Millipore, Billerica, MA). IgA, cyclin D1 and beta actin were detected using antibodies to IgA (Biosource, Camarillo, CA), cyclin D1 (Cell Signaling, Danvers, MA) or beta actin (Novus Biologicals, Littleton). In all cases the secondary Ab was horseradish peroxidase (HRP)-conjugated polyclonal anti-mouse or anti-rabbit IgG (GE Healthcare, Pittsburgh, PA). An enhanced chemiluminescence

detection reagent (Thermo Fisher Scientific Inc., Rockford, IL) and autoradiography were used to detect immunoreactive proteins.

Enzyme-linked immunosorbent assay (ELISA)

To quantitate Ig secretion, MC-B11/14^{WT} and MC-B11/14^{IgA-} cell lines were cultured in media containing 0.5% BSA with IL-6 (1ng/mL) at a density of 0.5x10^{6} cells/mL. Cell free supernatants (SN) were harvested after a 24 hr incubation. Microtiter plates were coated with the following antibodies; anti-kappa to measure total kappa (free and bound kappa) (Bethyl Laboratories Inc., Texas), anti-kappa FLC (Bethyl Laboratories Inc., Texas) to measure free light chain kappa or anti-IgA (Biosource, Camarillo, CA) to measure IgA. The plates were then blocked with phosphate-buffered saline containing 0.2% casein (BioFX Laboratories, Owings Mills, MD), washed and incubated with the cell-free SN for 2 hrs. Total kappa and free LC kappa were detected colorimetrically using anti-kappa-HRP (Bethyl Laboratories Inc., Texas), and IgA was detected using anti-IgA-HRP (Biosource, Camarillo, CA). The substrate used was O-Phenylenediamine dihydrochloride dissolved in stable peroxide substrate buffer (Pierce Chemical, Rockford, IL). Values were determined using a Molecular Devices (Sunnyvale, CA) microplate reader. Known amounts of purified human kappa LC and IgA (Jackson ImmunoReserach Labs, West Grove, PA) were used to generate standard curves. The assay detection limits ranged from 1μg/mL to 0.1ng/mL.

Annexin V Staining

Detection of annexin-V binding to apoptotic cells was performed using an Annexin-V–FLUOS staining kit (Sigma Aldrich, St. Louis, MO) and an Accuri flow cytometer (BD Biosciences, San Jose, CA).