Supporting information for Klein *et al.* (2003) *Proc. Natl. Acad. Sci. USA*, 10.1073/pnas.0437996100

Magnetic Cell Separation

The tonsillar specimens were kept on ice immediately after surgical removal. To prepare tonsillar MCs, tonsillar tissue was minced, the cell suspension was passed through a sieve, and tonsillar MCs were isolated by Ficoll-Isopaque density centrifugation. The four B cell subpopulations were isolated by magnetic cell separation using the MidiMACS system (Miltenyi Biotech). The purity of the isolated fractions was determined on a FACS Calibur (Becton Dickinson). All stainings, generally 10 min for mAbs and 20 min for microbeads (MB; all from Miltenyi Biotech), were performed in PBS, 0.5% BSA on ice; all magnetic cell separations were carried out in the cold room with ice-cold PBS/0.5% BSA. For the isolation of naïve B cells, tonsillar MCs were incubated with anti-CD27 and anti-CD10 (both Pharmingen). After washing with PBS/0.5% BSA, the cell suspension was stained with anti-IgG1-MB and anti-CD14-MB. Magnetically labeled cells were depleted by passing the cell suspension over a LD Column (Miltenyi Biotech). The flow-through was incubated with anti-IgD-FITC (Pharmingen), and in a second staining step with anti-FITC-MB. IgD-positive B cells were isolated by using LS Columns. The purity of the isolated fraction was determined by stainings with anti-IgD-FITC, anti-CD27-PE (Pharmingen), and anti-CD38-PE (Becton Dickinson). CB were isolated in a single step of magnetic cell separation by staining tonsillar MCs with anti-CD77 (Coulter/Immunotech) (CD77 represents a neutral glycolipid (globotriosylceramide; Gb3) that is not present in other cells), followed by incubation with mouse-anti-Rat-IgM (MARM; Pharmingen), and finally anti-IgG1-MB. The cells were passed over an LS Column with a G22 needle to reduce the flow rate, eluted from the column, and passed again over a second LS Column without needle. Purity was determined by staining against CD38, CD77 [revealed by MARM-FITC (Pharmingen) staining], and CD3 [anti-CD3-FITC (Coulter/Immunotech)]. For the

isolation of CC, tonsillar MCs were first incubated with anti-CD77, and after washing with PBS/0.5% BSA, anti-CD39 and anti-CD3 (both Coulter/Immunotech), and MARM. The cell suspension was subsequently stained with anti-IgG1-MB. The cells were passed over an LS Column with G21 needle; this step primarily resulted in the depletion of $CD77^+$ cells. The flow-through was subsequently passed over a LD Column to deplete the remaining magnetically labeled cells. The resulting flow-through was incubated with anti-CD10, and in a second step with anti-IgG1-MB. $CD10^+$ CCs were isolated by using LS Columns. Purity was determined by staining against CD38, CD3, and CD77. We observed that CD77-negative CC comprise only a small fraction in most tonsillar MCs. To obtain a high purity, we purified CC from tonsils that showed a higher than average percentage of CD77— GC B cells. For the isolation of memory B cells, tonsillar MCs were incubated with anti-CD10, anti-CD3, and anti-CD38 (Pharmingen), the latter at a titer of 1:1,000 to facilitate a selective depletion of $CD38^{high}$ plasma cells and GC B cells. After washing, the cell suspension was stained with anti-IgG1-MB and anti-CD14-MB. Labeled cells were depleted by passing the cell suspension over an LD Column. The flow-through was stained with anti-CD27-FITC, and in a second step with anti-FITC-MB. $CD27^+$ cells were isolated by using LS Columns. Purity was determined by staining for CD38 and CD27. Detailed protocols for the isolation of the four subsets are available on request.

Because tonsillar MCs are almost exclusively composed of B and T lymphocytes, the latter are the major potential source of cellular contamination in our isolation protocols. To exclude the possibility that in the DNA microarray analysis some of the genes defining the profiles actually correspond to message from contaminating T lymphocytes, we purified T cells from the tonsillar MCs of two individuals by using anti-CD3-MB. Comparing the corresponding gene expression data with those of the B cell subset-specific profiles, only a few genes were identified that potentially might have been derived from T cells. Macrophages/monocytes comprise only around 1% of tonsillar MCs. The occurrence of such cells in any of the four fractions is unlikely because message for macrophages/monocytes-specific genes such as *CD14* or *CD33* was not detectable on the DNA microarrays of all 20 hybridizations.

Amplification of c-Myc mRNA by PCR

Complementary DNA generated from RNA of the Burkitt lymphoma line Ramos, CB, naïve B-cells, and the HeLa cell line was amplified by PCR with the c-Myc sense and antisense oligonucleotides described by Martinez-Valdez *et al*. (1). PCR conditions were as described (annealing temperature 60°C, 35 cycles) and also varied by applying different Mg^{2+} concentrations, temperature, and by adding DMSO. The expected size of the corresponding PCR band is 988 bp. Amplification of the HeLa, Ramos, CB, and naïve B-cell cDNA mixtures yielded a band of ~430 bp on ethidium bromide-stained gels in all samples; the band was predominant in Ramos and less so in the CB, naïve B cells, and HeLa. A weak PCR band of the expected 988 bp could be detected only in the amplified Ramos and HeLa cDNA samples. Sequencing of the ~430-bp band revealed a product comprising 46 bp homologous to c-Myc exon 1 (italics) and 392 bp homologous to exon 2 (bold) with a 3-bp overlap; oligonucleotide sequences are underlined:

*GAGAGGCAGAGGGAGCGAGCGGGCGGCCGGCTAGGGTGGAAGA****GCC*****CTCCTA**
CGTTGCGGTCACACCCTTCTCCCTTCGGGGAGACAACGACGGCGGTGGC
GGGAGCTTCTCCACGGCCGACCAGCTGGAGATGGTGACCGAGCTGCTG
GGAGGAGACATGGTGAACCAGAGTTTCATCTGCGACCCGGACGACGAGA
CCTTCATCAAAAACATCATCATCCAGGACTGTATGTGGAGCGGCTTCTCG
GCCGCCGCCAAGCTCGTCTCAGAGAAGCTGGCCTCCTACCAGGCTGCGC
GCAAAGACAGCGGCAGCCCGAACCCCGCCCGCGGCCACAGCGTCTGCTC
CACCTCCAGCTTGTACCTGCAGGATCTGAGCGCCGCCGCCTCAGAGTGC
ATCGACCCCTCGGTGGTCTTCCCCTACCCTCTCAACGACA

This PCR product most likely represents a PCR artifact that because of its smaller size is preferentially amplified over the expected 988-bp product. A weak 988-bp PCR product was obtained from Ramos but not from the CB or naïve B cells. This finding is consistent with the observation that Ramos shows much higher mRNA levels for c-Myc than the

normal B cell subsets (Fig.3*A*). The discrepancy of these results compared to those reported by Martinez-Valdez *et al*. (1) is unclear.

1. Martinez-Valdez, H., Guret, C., de Bouteiller, O., Fugier, I., Banchereau, J. & Liu, Y. J. (1996) *J. Exp. Med.* **183,** 971–977.