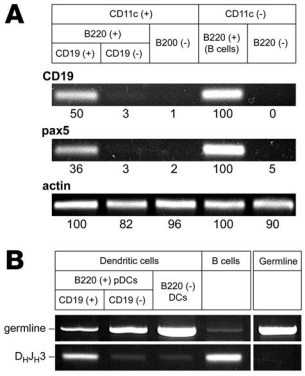


Supplemental Figure 1
Evidence of an early B-lineage link for CD19⁺ pDCs. (**A**) Cells from TDLNs were sorted as in Figure 6. RNA was analyzed by quantitative real-time RT-PCR. Gels show the expected product for CD19, pax5 and γ-actin after 27 cycles of amplification (linear range); each gel showed a single band. The numbers below each band give the mRNA quantitation by real-time RT-PCR, normalized to the γ-actin message for each fraction, and expressed relative to the authentic B cell fraction (arbitrarily defined as 100%). (**B**) $D_H \rightarrow J_H$ recombination in CD19⁺ pDCs. DCs from TDLNs were sorted as in the previous experiment, and DNA analyzed for DJ recombination at the IgH locus. The positive control was B cells ($B220^+CD11c^{NEG}$) from the same LN, and the negative (germline) control was tail-tip DNA. The PCR products corresponding to the germline configuration (upper band) and the 333 bp fragment produced by D_HJ_H3 rearrangement (lower band) are shown. (The same primers also amplified 741 and 1058 bp products corresponding to the $D_H \rightarrow J_H2$ and $D_H \rightarrow J_H1$ rearrangements, but the pattern was identical to the D_HJ_H3 band.

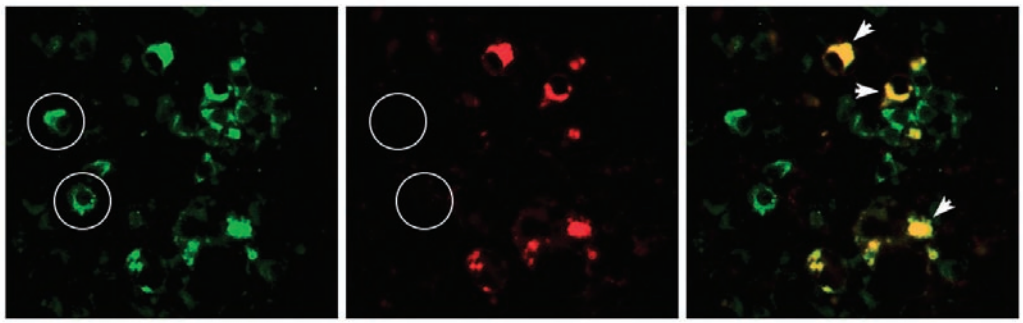
To confirm that the expression of CD19 was authentic, we measured mRNA for CD19 by quantitative RT-PCR following sorting of DC subsets. We also measured the transcription factor pax5, since expression of CD19 is obligately dependent on pax5 (1, 2). Supplemental Figure 1 shows that sorted CD19⁺ pDCs expressed mRNA for both CD19 and pax5. Quantitatively, these transcripts were expressed at lower levels than found in mature B cells, but they were much higher than in either the $CD19^{NEG}$ fraction of pDCs, or in the $B220^{NEG}$ DCs. Also consistent with derivation from a B-lineage or common-lymphoid precursor, the CD19⁺ pDCs showed a significant degree of early recombination at the IgH locus. Recombination was not as extensive as in authentic B cells (which showed essentially complete loss of the germline allele), but it was significantly greater than the $CD19^{NEG}$ pDCs, or the $B220^{NEG}$ DCs.

Methods

Quantitative RT-PCR and PCR for DJ recombination

Total RNA was extracted from MoFlo-sorted cells using Trizol (Gibco-BRL, Gaithersburg, Maryland, USA). RNA was reverse-transcribed and amplified using the LightCycler real-time PCR system (Roche, Pleasanton California, USA) with the RNA SYBR Green kit (Roche). All groups were compared in the same run, and quantitated against a standard curve of spleen RNA. Primers for mouse γ-actin were: sense=GATGACGCAGATAATGTTT, antisense=TCTCCTTTATGTCACGAAC (290 b.p. product). Primers for mouse CD19 were: sense=GGCACCTATTATTGTCTCCG, antisense=GGGTCAGTCATTCGCTTC (218 b.p. product). Primers for mouse pax5 were: sense=GCATAGTGTCTACAGGCTCCG; antisense=GATGGGTTCCGTGGTGGT (299 b.p. product). Conditions were optimized for each primer set to yield a linear standard curve over a 3-log range, with a correlation coefficient in every experiment of $r > 0.99$. An aliquot of each sorted sample was pre-screened to determine γ-actin message, then samples were loaded to amplify an equivalent amount of γ-actin. For gel electrophoresis, RT-PCR was performed for the pre-determined optimum number of cycles to yield amplification in the linear range, and products resolved on formaldehyde gels.

For gene rearrangement at the IgH locus, DNA was extracted from sorted LN cells using the QIAmp Micro kit (Qiagen), or from tail-tip biopsies using Trizol. The germline allele was detected using the Mu0 and J3 primers as described in ref. (3), which amplified a ⌈1400 bp product from the unrearranged locus. $D_H \rightarrow J_H$ recombination was detected using the DHL and J3 primers from the same reference, which amplified a ⌈333 bp product for the D_HJ_H3 rearrangement. Following PCR (TaKaRa Mirus Bio, Madison, Wisconsin, USA) products were resolved by agarose gel electrophoresis and visualized with ethidium bromide.



Supplemental Figure 2
Human tumor-draining LNs contain a subset of cells that co-express IDO and CD20. Melanoma sentinel LNs were stained for IDO (green) versus the B-lineage marker CD20 (red) by dual immunofluorescence. The figure shows separate green and red images of the same field, with the merged image at right. There were a number of cells with plasmacytoid-appearing morphology that expressed IDO, a subset of which also co-expressed CD20 (arrows, right-hand panel). Not all IDO⁺ cells expressed CD20 (circles).

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