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Adoptive Transfer of Murine B Cell Lineages

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Previous studies distinguished two murine B cell lineages: the conventional lineage, which contains cells that comprise the majority of B cells in spleen and lymph node of adult animals; and the Ly-1 B lineage (now called B-1 cells), which represents only a small percentage of the total adult B cell population and is principally found in the peritoneal and pleural cavities and at low frequency in the spleen[1]. Cell transfer experiments into lethally irradiated Igh-C allotype congenic recipients followed by multiparameter FACS analyses distinguish these B cell lineages[2]: conventional B cells are replenished from progenitors in adult bone marrow whereas B-1 cells are readily replenished by transfer of mature Ig^+ cells from the peritoneal cavity[3]. Further studies have added a third subset, the $CD5^-$ Ly-1 B cell "sister" population (now called B-1b cells), which shares most of the properties of the $CD5^+$ Ly-1 B cells (B-1a cells), including the characteristic ability to self-replenish[4] (also see Stall et al, these proceedings). Here we report further adoptive transfers with particular attention to the reconstitution of B-1b cells. Transfer of FACS sorted populations is used to distinguish progenitor activity from self replenishment.

Our B cell adoptive transfer studies with irradiated recipients and Igh-C allotype congenic mice are summarized for B-1a, B-1b, and conventional B cells, in the table. We find that B-1b cells derive from progenitors that persist into adulthood more readily than the progenitors that give rise to the B-1a cells (IgM^{br}, IgD^{lo}, $Ly\text{-}1^+$, and $Mac1^+$ in the peritoneum). B-1b cells, which are identified by the FACS phenotype IgM^{br}, IgD^{lo}, $Ly\text{-}1^-$, and $Mac1^+$ in the peritoneum, account for 10-30% of peritoneal B cells in normal, untreated, unirradiated animals. The B-1b progenitors are present in the $B220^-$ fraction of adult bone marrow . Progenitors for all three B cell populations are active in fetal liver, but progenitors for B-1a cells are largely missing or nonfunctional in adults.

In the Balb recipients, bone marrow donor-derived peritoneal B-1a cells range from barely detectable to 15% of normal. The cause of this variation is not well understood. However, it appears rare B-1a progenitors are present in the adult.

Mixed transfers of fetal liver and adult bone marrow prove that there are no accessory cells in the bone marrow which block B-1a cell development from the fetal liver; likewise, fetal liver does not enhance bone marrow reconstitution of B-1a cells. Additional evidence from the mixed transfers suggests independent development of B-1 cells and conventional B cells.

Adult spleen also contains the $B220^-$ B cells progenitors which reconstitute the three B cell populations in the same proportions as $B220^-$ progenitors from adult bone marrow. In addition, adult spleen contains IgM^+ B-1 cells capable of self replenishment. FACS sorted $B220^+$, IgM^+ spleen cells, which were injected with sorted $B220^-$ syngeneic bone marrow as a hematopoietic source, yield a low but consistent level of both B-1a and B-1b B cells in the recipient peritoneum. Conventional B cells (IgM^{lo}, IgD^{br}, $Mac1^-$, $Ly\text{-}1^-$ in the peritoneum or spleen) were not repopulated in the irradiated recipients. The presence of B-1b cells in the peritoneum of these recipients is further demonstration B-1b cells are present in the adult spleen. B-1 cells from spleen and peritoneum are equally efficient on a per cell basis at self-replenishment.

References

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	Donor Cells					
	Fet. Liv.	Bone Marrow		Spleen		PerC	
B Cell Lineage		$B220^-$	$B220^+$	$B220^-$	IgM^+	IgM^-	IgM^+
Conventional	+++	+++	-	+++	-	-	-
B-1b	+++	++	-	++	+	-	+++
B-1a	++	+/-	-	+/-	+	-	+++

Selective reconstituion of B cell Lineages. The reconstitution score is based on the average return of each B cell population relative to its normal level in the mouse peritoneum and spleen: +++ = >70% of normal, ++ = 30-70% , + 10-30%, +/- = 3-10%, - = <3%. The number of cells transferred equals the numberof cells of each phenotype found in 10^6 fetal liver (d 14) cells, $2x10^6$ adult bone marrow, $2x10^7$ adult spleen or $3x10^6$ adult peritoneal cells