Old Herborn University Seminar Monograph 6:
The ontogeny of the immune system.
Editors: Peter J. Heidt, Dan Holmberg, Volker Rusch, and Dirk van der Waaij.
Herborn Litterae, Herborn-Dill, Germany: 20-32 (2003).

WHERE DO CD5 B CELLS COME FROM?

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The definition of the "$CD5^+$ B cell population" in mice is loosely based on the presence of any of several attributes including: expression of CD-5 and/or CD11b, the absence of CD23, low CD45R(B220), high CD44, low Igδ, increased size and decreased density, increased plastic adherence, a limited repertoire of immunoglobulins, unusual tissue distribution, long-life and/or self-renewal capacity, and foetal origin (*Hayakawa* and *Hardy*, 1988; *Herzenberg* et al., 1986). For instance, a recent proposal holds that a population of B cells (called B-1 cells) arise early in ontogeny, are self-replenishing, absent in the adult marrow and lymph nodes, predominate in the pleural and peritoneal cavities and make up only a small fraction of splenic B cells (*Allison*, 1991). The majority of these cells (the B-1a population) express CD5 and the minority (B-1b) do not. The proposal goes on to term all other B cells as the B-2 population.

The contention of this review is that this attempt at a consensus definition fails. What if B cells of adult origin become $CD5^+$? Are all CD5 expressing cells B-1a cells? Are all peritoneal $CD5^+$ cells of foetal origin? Are B-1b cells of foetal origin? With these and related questions in mind I will present preliminary results that question the proposition that only B cells of foetal origin can become "CD5 B cells." After briefly reviewing other work addressing the issue of differences between B cells of adult and foetal origin I suggest an alternative view - the specificity hypothesis - of the origin of CD5 B cells is necessary. Finally, I discuss recent published studies from several laboratories with immunoglobulin transgenic mice that provide evidence for this second view.

Previously we asked if adult splenic B cells could be induced to express CD5. We thought that activation induced by the cross-linking of sigM would be likely to accomplish this. Indeed, that is precisely what we found (*Ying-Zi* et al., 1991). We showed that goat anti IgM as well as monoclonal rat anti IgM induced CD5 expression. We showed that this induction began within eighteen ours and involved all B cells by four days. We also showed that even selected $CD5^-$ B cells could be so induced.

This induction results in an increase in processed CD5 mRNA as detected by PCR amplification from cDNA. (Preliminary studies of Ram Bandyopadhyay and Henry H. Wortis.) We have not found sufficient message in freshly isolated $CD5^+$ peritoneal or *in vitro* induced splenic cells for detection by Northern blotting. At the present time we do not know if the accumulation of processed CD5 mRNA results from increased transcription, the onset of processing or message stabilisation. Studies to establish this are currently underway.

In recent preliminary studies (Chris Huang, John Iacomini, Thereza Imanishi-Kari and Henry H. Wortis) we asked whether B cells of adult origin could become $CD5^+$. Other groups have reported that $CD5^+$ cells are derived from adult bone marrow transfers

Table 1: Features of B cells derived from foetal and adult precursors

Feature	Foetal	Adult	Reference
$CD5^+$	many	few	*Riggs* et al., 1990 *Thomas-Vaslin* et al., 1992 *Iacomini* and *Imanishi-Kari*, 1992 *Hardy* et al., 1991, 1992 *Solvason* et al., 1991
$CD11b^+$	some	some	*Kantor* et al., 1992
$CD5^-CD11b^-$	few	many	*Riggs* et al., 1990 *Thomas-Vaslin* et al., 1992 *Iacomini* and *Imanishi-Kari*, 1992 *Hardy* and *Hayakawa*, 1991 *Hardy* et al., 1991, 1992 *Solvason* et al., 1991
Peritoneal	many	few	*Riggs* et al., 1990 *Thomas-Vaslin* et al., 1992 *Iacomini* and *Imanishi-Kari*, 1992 *Hardy* and *Hayakawa*, 1991 *Hardy* et al., 1991, 1992 *Solvason* et al., 1991
Self-renewing	many ?	few ?	not reported
N-less	many	few	*Gu* et al., 1990 *Feeney*, 1990 *Carlsson* and *Holmberg*, 1990 *Bangs* et al., 1991 *Meek*, 1990
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TdT [terminal Deoxynucleotidyl transferase] (pre-B)	few	many	*Desiderio* et al., 1984
mlc-2 [myosin light chain-2] (pre-B)	none	all	*Oltz* et al., 1992

(*Riggs* et al., 1990; *Thomas-Vaslin* et la., 1992; *Iacomini* and *Imanishi-Kari*, 1992), but this has not been a universal experience (*Hardy* and *Hayakawa*, 1991). The sources of these differences have not been studied in detail, but there are three important considerations. If the frequency of potential $CD5^+$ cells is much less in the marrow than in the foetal liver then early examination of the recipients (e.g. after three months) might fail to reveal cells of bone marrow origin. Conversely, if the bone marrow preparation is contaminated by self-renewing cells of foetal origin a false positive result might be obtained.

In our experiments we transferred adult bone marrow from C57BU6 mice into sublethally irradiated C3H.SCID mice. Ten months later we found that a fraction of the splenic B cells were $CD5^+$. We placed B cell enriched splenic cells from the C57BU6 into SCID mice into *in vitro* culture with either LPS or anti-lg. We harvested the cells and double-stained them with FITC anti CD45(B220) and biotinylated anti CD5 plus phycoerythrin streptavidin and analysed them by flow cytometry. All of the anti Ig stimulated cells became $CD5^+$ while few, if any, of the LPS stimulated cells did so.

To determine whether these $CD5^+$ B cells were truly of bone marrow origin (and not derived from a few passenger cells of foetal origin) we took advantage of the observation that B cells of foetal/neonatal origin lack N sequences in their rearranged heavy chains while cells of adult origin have N insertions. We asked whether the junctional sequences of the rearranged heavy chains of these $CD5^+$ B cells reflected an adult or foetal/neonatal origin.

We examined the junctional sequences of rearranged genes containing members of the S107 V_H gene family. We did this because this family is believed to contain only four germ-line V_H genes and the sequences for these genes in the closely related C57BU10 strain (as well as the BALB/c strain) are known. This knowledge makes assignment of N sequences in VD junctions possible. Our strategy was to generate cDNA and use it as template for PCR based amplification using a 5' primer for V_H genes of the S107 family and a 3' CH1 cμ primer. Thus far, all of the heavy chain sequences that we have examined contain N sequences. We conclude that $CD5^+$ B cells can be derived from adult bone marrow.

Table 1 summarise results of studies on the origin of B cells expressing selected phenotypic features. This list is limited to studies in which an attempt was made to exclude the possibility that mature B cells were included in the "progenitor population".

From these published data it is evident that only one of the examined traits, *mlc-2* expression, might be a definitive lineage markers of foetal or adult derived mature B cells. The product of the gene encoding a myosin light chain (*plrlc*), a member of the *mlc-2* family of proteins, is limited in expression to pre-B cells of adult origin (*Oltz* et al., 1992). However, this gene is not expressed in mature B cells. The table also makes it clear that frequencies of expression of several traits do differ markedly between cells of adult and foetal origins. What the data do not tell us is whether cells are predetermined at the progenitor level to express a given mature phenotype.

Pre-B cell surface antigens that are candidate lineage specific molecules are listed in Table 2. The two obvious candidates, CD5 and CD11b do not appear on freshly isolated sig cells nor are they uniformly expressed on Abelson-MuLV transformed pre-B cells derived from either foetal or adult compartments (unpublished results) and therefore are not markers of a lineage *per se*. The absence of CD45RA and Qa-2 was noted in pre-B cells of early embryos. Because foetal B cells appear to develop in a single wave (*Strasser* et al., 1989) it is possible that more primitive B lineage cells were sampled and these cells did not yet express these markers. Several years ago it was reported that the combination of lack of Thy-1 expression and presence of surface asialo-ganglio-N-tetraosylceramide (aGM,) was unique to foetal progenitors of B cells (*Hardy* et al., 1987). However, foetal cells with this phenotype did not yield particularly high numbers of peritoneal $CD5^+$ B cells.

Typical peritoneal $CD5^+$ cells have several characteristic phenotypic features, which suggested to us that they are activated B cells. They are large, low density cells with high levels of CD44 with little or no IgD that express IL-5 receptors (*Hitoshi* et al., 1990). We looked for some of these features in our *in vitro* induced $CD5^+$ cells. We found that become large cells with elevated CD44 without losing IgD (*Ying-Zi* et al., 1991). We speculated that in addition to sig cross-linking another signal, perhaps provided by a cytokine might be necessary. We tested IL-1 (with and without IL-4), IL-2, IL-4

alone and IL-6 as candidate interleukins. In our hands only IL-6 induced a loss of IgD, leading to the speculation that *in vivo* the combination of sig cross-linking and IL-6 induces the classic peritoneal $CD5^+$ phenotype. IL-5 receptors are reported to be induced on B cells following sigD cross-linking (although CD5 induction was not seen in this study) (*Allison* et al., 1991).

It is now generally accepted that the frequency of N insertions into rearranged heavy chains differs in B cells of foetal/neonatal and adult origin. The differences between the two populations are not absolute, and N-less segments have been found in cells of adult origin, although with low (2-10%) frequency, while only rarely were N insertions seen in foetal B cells (0.5-2%) (*Feeney*, 1992). This difference in N insertion pattern correlates with differences in the expression of terminal deoxynucleotidyl-transferase (TdT) (*Desiderio* et al., 1984).

The finding that the addition of IL-6 to sig cross-linked B cells induces many of the features of the CD5 B cell is provocative because it means that B cell activation in the absence of T cells can result in the induction of the CD5 phenotype. IL-6, which is produced by T helper-2 cells, is also made by fibroblasts (*Zilberstein* et al., 1986), macrophages (*Aarden* et al., 1985), plasma cells (*Kawano* et al., 1988) and endothelial cells. Therefore, *in vivo* $CD5^+$ B cells might be generated by multiple epitope (repeating unit) antigens, the thymus-independent type 2 (Tl-2) antigens, in association with IL-6. Because antibody responses to Tl-2 antigens require macrophages it is reasonable to hypothesise that CD5 cells result from Tl-2 antigen presentation by macrophages or other IL-6 producing cells.

B cells activated by anti-lgM and IL-6 also lost their surface CD23 and had decreased amounts of CD45(B220) IgD (*Ying-Zi* et al., 1991), both features of peritoneal $CD5^+$ B cells. Apparently then, CD23 loss could result from activation. We thought that we might be able to find examples of B cells at a point after activation *in vivo* at which they expressed CD5 but had not yet lost their CD23. It was likely that cells with this phenotype might be more prevalent in the young mouse, at a time when there are many splenic CD5 cells. We reasoned that these would be newly activated cells that might not yet express a mature phenotype. Indeed, when we examined small $CD5^+$ B cells from young mice we found them to be $CD23^+$ (*Rabin* et al., 1992a). We think it is reasonable to propose that many B cells of foetal/neonatal origin are stimulated by Tl-2 type antigens to enter the CD5 pathway, subsequent ligation of IL-6 (or an equivalent cytokine) inducing the loss of IgD and CD23 and the characteristic secretion of IgM and IgG_3.

We also noted that losses of CD23 (*Kikutani* et al., 1986) and IgD (cf, *Kroese* et al., 1990) together with a decrease in CD45RB (*Birkeland* et al., 1988) are seen in B cells within germinal centres where CD5 is not expressed. Activation of B cells within germinal centres is a T cell dependent process. This suggests that T cell dependent activation of B cells might produce cells with a characteristic surface phenotype. We were encouraged to think along these lines by our observation that activation with LPS failed to induce CD5 expression but did cause increased CD44, as well as the loss of CD23 and IgD. Interestingly, LPS induced a dramatic decrease in the expression of the heat stable antigen (HSA) as detected by the monoclonal antibody J11d. We had not seen J11d loss after treatment with anti IgM, even with added IL-6, nor was J11d low on unmanipulated peritoneal $CD5^+$ B cells.

Table 2: Surface markers of pre-B cells

Molecule	Foetal	Adult	Reference
CD45	yes	yes	*McKearn* et al., 1985
CD45RA	No/yes	yes	*Kincade* et al., 1981 *Landreth* et al., 1983 *Paige* et al., 1985
CD45R(B220)	yes	yes	Many, cf. *Strasser*, 1988
Qa-2	no	yes	*Kincade* et al., 1981
CD72 (Lyb-2)	no/yes	yes	*Kincade* et al., 1981 *Paige* et al., 1984, 1985
PB76	yes	yes	*Strasser*, 1988
AA4.1	yes	yes	*Paige* et al., 1985 *McKearn* et al., 1984
GF1	yes	yes	*McKearn* et al., 1984, 1985
BP-1	yes	yes	*Strasser*, 1988 *Cooper* et al., 1986
CD2	?	yes	*Yagita* et al., 1989a,b
HAS	yes	yes	*Hardy* and *Hayakawa*, 1991, 1992 *Hardy* et al., 1991
[Heat stable antigen]			(unpublished)
CD43	yes	yes	*Hardy* and *Hayakawa*, 1991, 1992 *Hardy* et al., 1991

We then tested the idea that B cells could be activated by two distinct induction pathways. One, the CD5 inducing pathway has been discussed above. The other, we thought, would occur after T dependent *activation without the need for slg cross-linking*. We (David Parker and Henry H. Wortis) are currently using a model system involving activation with purified rabbit anti mouse IgM. We stimulate high density resting splenic B cells in one of two ways: with $F(ab')_2$ of rabbit anti mouse IgM antibody; or with Fab of the same antibody together with equal numbers of a T helper cell line specific for rabbit Fab. Our early, preliminary results indicate that both treatments result in activation as measured by greater forward and size scatter and increased CD44 expression. Only the cells stimulated by F(ab')2 became $CD5^+$. Only the cells stimulated by Fab plus T helper cells became $J11d^{low}$. Control groups cultured with Fab or T helper cells alone did not become activated nor altered in surface phenotype.

Previously David Parker demonstrated that B cells can be activated by cognate interaction with helper T cells even in the absence of sig cross-linking (*Tony* et al., 1985). Subsequent work has amply confirmed this idea. This finding suggested the possibility that there are two distinct signals for B cell activation, each inducing a unique differentiation pathway. Current evidence is entirely consistent with the idea that $CD5^+$ B cells are cells that have responded to Tl-2 antigen stimulation but not definitive. Features distinguishing TD and Tl-2 responses are summarised in Table 3.

It is possible that not all B cells are able to enter the TD pathway. Norman Klinman suggests that only B cells with a $J11d^{low}$ phenotype can enter the memory pool and undergo affinity maturation (*Linton* et al., 1989). Since then it is was shown that low J11d can be in-

Table 3: Differences in B cell responses to thymus dependent and thymus independent-2 stimuli

	Type of stimulation		
Feature	TD	Tl-2	Reference
Surface	$CD5^-$	$CD5^+$	Proposed
Phenotype	$J11d^{low}$	$J11d^{high}$	Proposed
Early activation gene *egr*-1 expressed	no	yes	*Klaus* and *Parker*, 1992
Isotypes	IgM, IgG^1, IgG^2 IgA, IgE	IgM, IgG^3	*Slack* et al., 1980
In germinal centers	yes	no	*De Sousa* et al., 1969 *MacLennan* et al., 1982
Somatic mutation	common	rare	cf. *Rajewsky* et al., 1987
Memory	yes	no	*Howard* and *Courtenay*, 1974

duced by IL-4 (*Yin* and *Vitetta*, 1991; and our own unpublished observations) or by LPS IgD (*Ying-Zi* et al., 1991). Therefore, low J11d must not be a definitive marker for a predetermined a lineage of memory B cells. Nevertheless, Klinman has shown that only transferred $J11d^{low}$ cells generate high affinity antibodies (*Linton* et al., 1992). He argues that since these cells are found in nude mice they are unlikely to originate only from an initial interaction with helper T cells. Recently it was shown that both T helper 1 and 2 cells can induce affinity maturation (*Rizzo* et al., 1992). It is not known whether Th1 cells induce expression of the $J11d^{low}$ phenotype.

If CD5 B cells do not generate somatic mutations (but see *Taki* et al., 1992), there are important implications for our understanding of the origin of pathogenic autoantibodies. Early B cells express a repertoire that recognises conserved epitopes including self- antigens. Since this is a germ-line encoded repertoire (not requiring junctional diversity) it has been selected over evolutionary time. The precise selective advantage conferred by this repertoire is uncertain. It may provide for a first line of defence against pathogens, substitute for regulatory molecules, or regulate the antibody response. None of these are mutually exclusive properties.

That the foetal repertoire is skewed toward binding many epitopes, but with low affinity, makes it likely that early B cells will be activated by self-antigens. The reason for this is shown in Figure 1. Here it can be seen that sig cross-linking will be readily induced by repeating unit antigens. On the other hand, unique epitopes, such as those found on most protein antigens will fail to accomplish this. Finally, if a cell surface presents multiple unique epitopes which can all be recognised by the polyreactive antibody of a given B cell, that B cell might be induced by sig cross- linking to become $CD5^+$ and secrete IgM antibody. This makes it highly likely that the emerging foetal/neonatal repertoire will be activated by contact with self-antigens. Adult B cells, expressing antibodies with high affinities for unique epitopes would not be likely to be triggered in this manner.

Some time ago the ability of $CD5^+$ B cells to provide help for antibody responses was reported (*Okumura* et al., 1982; *Sherr* and *Dorff*, 1984). The finding that CD5 B cells can produce IL-10, a viability factor for B cells, and

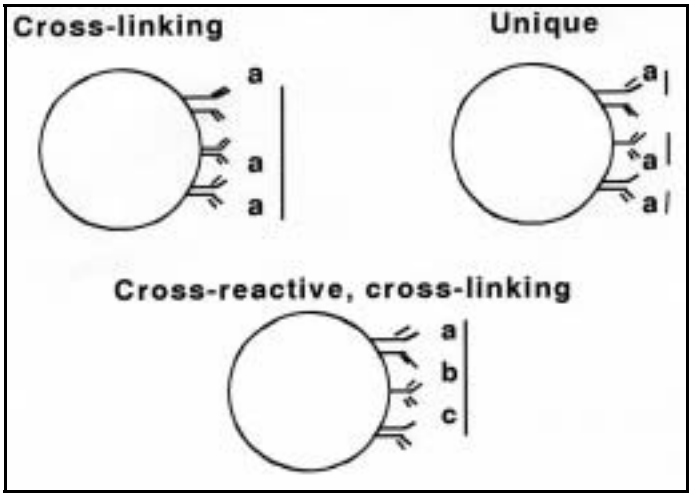

Figure 1: Patterns of B cell binding: upper left, binding to repeating unit antigen; upper right, binding to unique epitope antigen; bottom, polyspecific binding to multiple epitopes.

that B cells can produce other interleukins, such as IL-6, as well as TNF-α and -β (*O'Garra* et al., 1991) suggests a mechanism for B-B help. The finding that the ligand for CD5 is CD72 (Ly-b2) (*van de Velde* et al., 1991), itself a molecule expressed on B but not T cells suggests that CD5-CD72 ligation might play a role in this help (*Kamal* et al., 1991). Indeed, there is evidence that antibodies to CD72 block Tl-2 as well as TD antibody responses (*Subbaro* and *Mosier*, 1982). In any case, Ig-lg binding together with CD5-CD72 binding might provide the basis for B-B help.

We have proposed that $CD5^+$ B cells are Tl-2 responding cells rather than the product of a predetermined CD5 lineage. This "specificity" hypothesis is tested by immunoglobulin transgenic mice. It predicts that transgenic mice constructed to express an immunoglobulin with a specificity common to naturally occurring $CD5^+$ cells will contain a high frequency of $CD5^+$ cells. Confirmation was obtained in experiments utilising a transgene encoding a heavy chain including the V_H 11, D and J_H gene segments from a peritoneal B cell producing antibody with anti bromelain treated mouse red blood cell specificity (*Hardy* and *Hayakawa*, 1991; *Hardy* et al., 1991). A striking result was obtained with a heavy/light chain transgenic expressing an NZB derived anti mouse red blood cell autoantibody (*Okamoto* et al., 1992). Essentially all peripheral B cells are lost and only the peritoneal B cells remain. (However, the peritoneal B cells have not been directly tested for CD5 expression.) The proposed explanation of this result is that there is autoantigen induced deletion of the peripheral B cells and the few cells that make it to the peritoneum live on in a sequestered environment. An alternative interpretation of these experiments is that the accumulated CD5 lineage cells derive from foetal/neonatal precursors. This does not appear likely because there do appear to be transgene encoded antibody producing B cells in the marrow. A direct test of the specificity hypothesis would be a transfer of B cell precursors from the adult bone marrow of transgenic mice into SCID, RAG-1 or RAG-2 knockout mice.

The distortion of the endogenous

immunoglobulin repertoire seen in some immunoglobulin transgenics also provides evidence for the repertoire hypothesis. In the M54 mice the transgene encodes a heavy chain expressing a member of the J558 V_H family (17.2.25) (*Weaver* et al., 1986). The repertoire of B cells expressing endogenous genes is skewed toward Q52 and 7183 family members (*Iacomini* et al., 1991) with a high frequency of autoantibody production (*Thereza Imanishi-Kari*, personal communication). There appears to be activation and positive selection of these cells (*Grandien* et al., 1991; *Rabin* et al., 1992b). Further, there is an increased frequency of $CD5^+$ B cells within this B cell population (but not in those B cells expressing the transgene). Foetal cells do not appear to be the source of these B cells, as judged by transfer experiments and the presence of N insertions in DJ_H junctions (*Iacomini* and *Imanishi-Kari*, 1992). Therefore, in the M54 mouse the presence the 17.2.25 transgene causes a distortion of the endogenous repertoire favouring D proximal V_H gene utilisation. Autoantigens activate these cells causing an increase in the CD5 population.

An interesting phenomenon was observed when the 17.2.25 transgene was introduced into mice expressing *X-linked immune deficiency (XID)* (*Rabin* et al., 1992b). This defect causes a deficit of CD5 cells and a failure to respond to Tl-2 antigens. XID transgenic mice have normal numbers of transgene expressing cells but lack cells expressing endogenous immunoglobulin. Another transgenic mouse, which carries genes encoding the M167 immunoglobulin, produces B cells with specificity for PC (*Kenny* et al., 1991). This particular specificity occurs frequently in the anti PC repertoires of normal mice but is never seen in XID mice. Introduction of the M167 transgene into XID mice results in progeny that lack peripheral B cells expressing the transgene. This is not due to a failure to produce B cells as mature cells can be found in the bone marrow. Kenny and co-workers propose that the cells are lost to a deletion mechanism following activation via ligand binding. These results appear to confirm a model of B cell deletion in XID mice proposed many years ago by *Klinman* et al. (1983).

Proof of the predetermined lineage hypothesis may rest on the identification of a stable lineage marker. Until then, the weight of evidence supports the specificity hypothesis of CD5 B cell formation.

ACKNOWLEDGEMENT

This work was supported in part by NIH grant Al15803 and American Cancer Society Grant IM61764.

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