

## A DUAL ORIGIN FOR IgA PLASMA CELLS IN THE MURINE SMALL INTESTINE

Frans G.M. Kroese,<sup>1</sup> Willem A. Ammerlaan,<sup>1</sup> Gerrit Jan Deenen,<sup>1</sup> Sharon Adams,<sup>2</sup> Leonore A. Herzenberg,<sup>2</sup> and Aaron B. Kantor<sup>2</sup>

<sup>1</sup>Department of Histology and Cell Biology, Immunology Section, University of Groningen, Groningen, The Netherlands; and <sup>2</sup>Department of Genetics, Beckman Center, Stanford University, Medical Center, Stanford, CA, USA

### INTRODUCTION

More than two decades ago, Craig and Cebra<sup>1</sup> showed that Peyer's patches are an important source of progenitor cells for intestinal IgA plasma cells. The vast majority of B cells in Peyer's patches are conventional B cells, which are produced throughout the life of the animal and which are responsible for high-affinity antibody responses to a variety of antigens. More recently, we provided evidence that probably also B-1 cells (previously called Ly-1 or CD5 B cells<sup>2</sup>) also contribute significantly to the population of IgA plasma cells in the gut, at least in B lineage chimeras.<sup>3,4</sup> B-1 cells are almost absent from Peyer's patches and are enriched in the peritoneal cavity. These cells are largely self-replenishing and have a selected antibody repertoire with specificities frequently directed towards "natural antigens", autoantigens and bacteria-related antigens.<sup>5,6</sup> In studies presented here we provide additional data, both from transfer studies with sorted B-1 cells and from analysis of  $\mu, \kappa$  transgenic mice, to support our hypothesis that B-1 cells can contribute to the IgA response of the gut.

### STUDIES WITH B LINEAGE CHIMERAS

In previous experiments B lineage chimeras were constructed by reconstituting lethally irradiated mice with syngeneic bone marrow (BM) and peritoneal cells (PerC) from immunoglobulin allotype congenic donors.<sup>3</sup> Flow-cytometry analysis (FACS) shows that conventional B cells express the BM-donor Ig allotype and B-1 cells express the PerC donor Ig allotype. Since many (40%) IgA plasma cells in the gut express PerC-donor allotype, even up to one year after transfer, these cells most likely belong to the B-1 cell lineage. However, it is possible that long-lived and/or self-replenishing IgA+ memory cells are present among the PerC and expand in the (irradiated) recipient. FACS analysis indicates that some IgA+ cells are present in PerC, although their numbers are very low (approximately 1% of PerC in 10 week old BALB/c mice<sup>3</sup>). Therefore, we have now sorted IgA- B-1 cells (defined by their low levels of sIgD and high levels of sIgM) by three

color FACS and transferred these cells into Ig congenic lethally irradiated mice, together with syngeneic bone marrow. FACS analysis shows that in these chimeric mice (3 months after transfer), B-1 cells express the Ig allotype of the transferred sorted IgA- B-1 cells. Immunoperoxidase and fluorescence staining of gut sections from these mice demonstrates that high numbers of IgA plasma cells in the lamina propria express the IgA allotype of the sorted B-1 cells. Thus, these experiments show that sorted IgM<sup>high</sup>IgD<sup>low</sup> peritoneal B-1 cells are able to undergo isotype switching to IgA-expressing cells and to migrate to the gut lamina propria.

### IgA PLASMA CELLS IN THE GUT OF $\mu,\kappa$ TRANSGENIC MICE

The introduction of a functionally rearranged  $\mu$  heavy chain can perturb the function and development of B cells<sup>7-11</sup> and may reflect existing differences between the two B cell lineages.<sup>12-14</sup> In most transgenic mice the vast majority of B cells express transgenic IgM exclusively, as expected by the principle of allelic exclusion. A small population of the B cells, however, is still able to rearrange and express endogenous Ig genes demonstrating that allelic exclusion is not absolute. FACS analysis and transfer studies with M54 transgenic mice, containing a rearranged NP-specific  $\mu$  heavy chain transgene, have shown that expression of endogenous IgM is largely restricted to the B-1 cell lineage.<sup>12,14</sup> We have recently studied<sup>15</sup> B6-Sp6 mice which carry fully rearranged (BALB/c derived, Igh-C $\alpha$  allotype)  $\mu$  heavy chain and  $\kappa$  light chain transgenes, specific for TNP, on a C57Bl background (Igh-Cb).<sup>8</sup> Three criteria demonstrate that endogenous Ig is largely restricted to the B-1 lineage in B6-Sp6 mice. First, FACS analysis shows that the vast majority of B cells in peripheral lymphoid organs and bone marrow of B6-Sp6 mice express transgenic IgM exclusively (Fig. 1). Only a small proportion of B cells (<10%) in these tissues express endogenous IgM, usually simultaneously with the transgenic IgM. Endogenous IgM<sup>+</sup> cells in B6-Sp6  $\mu,\kappa$  transgenic mice are, however, clearly enriched in the peritoneal cavity (Fig. 1). Thus, endogenous IgM<sup>+</sup> cells have the same anatomical distribution as B-1 cells. Almost half of the peritoneal B cells express endogenous IgM, two-thirds concomitant with transgenic IgM. Second, the peritoneal endogenous IgM<sup>+</sup> cells display the B-1 cell phenotype. They express Mac-1 (CD11b) and low levels of IgD (Fig. 1) and many also express Ly-1 (CD5). Third, B6-Sp6 BM poorly reconstitutes endogenous IgM<sup>+</sup> cells, just as adult BM poorly reconstitutes B-1 cells. In contrast, B6-Sp6 BM reconstitutes transgene expressing B cells very well, just as normal BM reconstitutes conventional B cells very well. The few endogenous IgM<sup>+</sup> cells in the reconstituted mice are predominantly located in the peritoneal cavity and have the phenotype of the CD5<sup>-</sup> B-1b cells (Ly-1 B sister cells). Likewise, normal adult BM reconstitutes B-1b cells better than B-1a cells.<sup>16</sup> Together, these three criteria (anatomical localization, phenotype and relative BM independency) are sufficient to qualify the large majority of endogenous IgM<sup>+</sup> cells in  $\mu,\kappa$  transgenic B6-Sp6 mice as B-1 cells.

Immunohistological staining of the small intestine of B6-Sp6 mice shows the presence of high numbers IgA-containing cells (plasmablasts/-cells), and relatively few IgM-containing cells.<sup>15</sup> This IgM is exclusively transgenic IgM. Two-color immunofluorescence staining (IgM and IgA) of cytopins prepared from isolated lamina propria cells demonstrates three subsets of plasmablasts/- cells: a majority population of cells containing only IgA, one-third of the cells containing simultaneously IgA and (transgenic) IgM and a very small population of cells containing only (transgenic) IgM (Fig. 2). This IgA in the gut lamina propria is produced by endogenous  $\kappa$  immunoglobulin genes, since the transgene in the B6-Sp6 mice encodes only for a  $\mu$  heavy chain gene. Furthermore, two-color staining of gut sections from these mice with anti-IgA or anti-IgM in combination with a Mab directed to the transgenic idiotype (Id) shows that most IgA containing cells do not express the transgenic Id; only IgM containing cells (with or without IgA) are Id<sup>+</sup>. Thus, this IgA is the result of the complete, functional VDJ rearrangement.

Figure 1. Various sorted anti-Igh-6b avidin conjugated dead cells

Figure 2. Transgenic immunofluorescence followed by

isotype switch or trans-re generation Ap Forni<sup>19</sup> and cells produced

### Expression of endogenous IgM in B6-Sp6 $\mu, \kappa$ transgenic mice

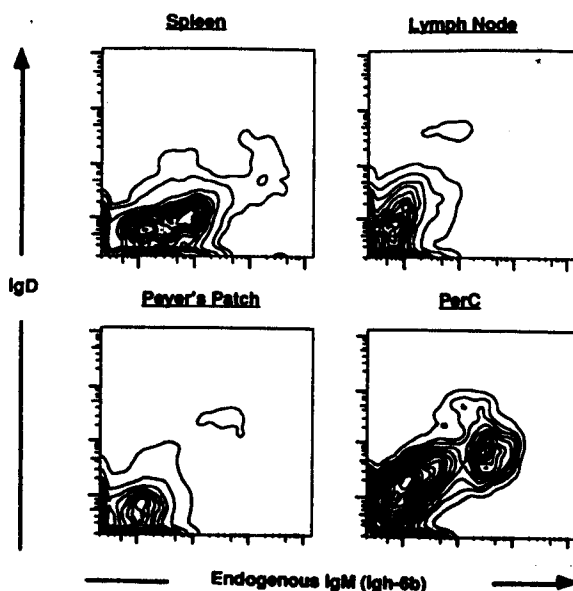


Figure 1. Expression of endogenous IgM and IgD on B cells from B6-Sp6 transgenic mice. Cells from various sources were stained and analysed by FACS for endogenous IgM and IgD using fluorescein-labeled anti-Igh-6b (AF6-78.25) and biotinylated anti-Igh-5b (AF6-122.2), respectively. Biotin was revealed by avidin conjugated to Texas red. Only lymphocytes are shown after gating on forward and obtuse scatter and dead cells were excluded by their staining for propidium iodide. Plots display 5% probability plots.

### Antibody containing cells in the gut lamina propria of B6-Sp6 $\mu, \kappa$ transgenic mice

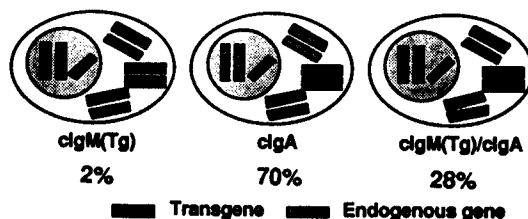


Figure 2. Schematic drawing of the types of plasma cells found in the gut lamina propria of B6-Sp6  $\mu, \kappa$  transgenic mice. Cytospins from isolated lamina propria cells were stained by two color immunofluorescence for fluorescein-conjugated anti-IgA (Mab 71.14) and biotinylated anti-IgM (Mab 331), followed by avidin-TRITC. At least 300 antibody-containing cells were analyzed.

isotype switching and expression of endogenous Ig genes, and is not due to trans-splicing<sup>17</sup> or trans-recombination<sup>18</sup> between endogenous heavy chains and the transgenic VH gene.

Apparently, the relatively few endogenous IgM<sup>+</sup> cells are responsible for the generation of many IgA-secreting cells in the gut. These data are consistent with findings of Forni<sup>19</sup> and Grandieu *et al.*<sup>20</sup> who observed that a large proportion of the plasmablasts/cells produce (endogenous) IgG and IgA. Our studies with M54<sup>12,14</sup> and B6-Sp6

transgenic mice<sup>15</sup> demonstrate that endogenous IgM<sup>+</sup> expression is almost exclusively confined to the B-1 cell population. As we summarize in Fig. 3, we conclude that the IgA antibody-containing cells in the gut lamina propria (and the IgG- and IgA-containing cells in the spleen) of these transgenic mice belong to the B-1 cell lineage and are derived from isotype switched IgM<sup>+</sup> B-1 cells. Why, endogenous IgM expression is largely restricted to

Most plasma cells in the gut of B6-Sp6 $\mu,\kappa$  transgenic mice belong to the B-1 cell lineage

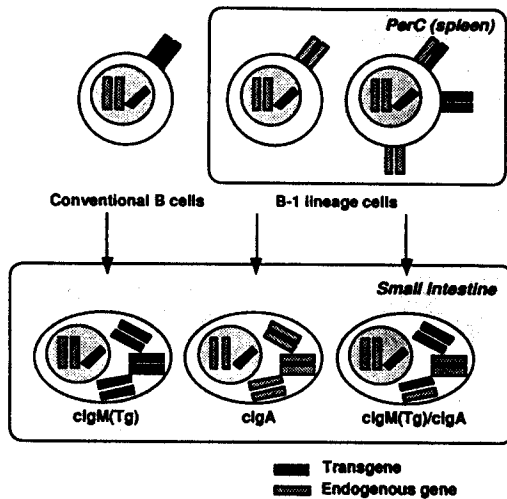


Figure 3. Model of the origin of IgA-containing cells in the lamina propria of the small intestine of B6-Sp6 $\mu,\kappa$  transgenic mice. For explanation see text.

the B-1 cell population is not known, but might be due to differences in development and/or selection mechanisms between B-1 cells and conventional B cells<sup>14</sup>.

### KINETICS OF B-1 CELLS

Approximately 15 million IgA-secreting cells are located in the murine gut lamina propria, and these cells account for 90% of all antibody-secreting cells in the mouse.<sup>21</sup> The majority of the IgA-containing cells in the lamina propria are short-lived cells with an estimated half-life of 5 days.<sup>22</sup> This means in absolute numbers that about 1.5 million IgA plasma cells are renewed daily. Given the low overall numbers of B-1 cells in the mouse (roughly 7-10 million cells<sup>6</sup>, and the observation that B-1 cells may be responsible for 40% of the IgA cells in the gut, this implies that B-1 cells must expand somewhere in the animal to produce enough IgA precursor cells. To address this point we have studied the kinetics of peritoneal B-1a cells in mice.<sup>23</sup> Metaphase arrest using vincristine sulphate and S-phase index labelling studies using a single injection of 5'-bromo-2'-deoxyuridine (BrdU) show that B-1a cells do not divide significantly within the peritoneal cavity. Long term oral administration of BrdU in combination with three color immunocytology demonstrates that peritoneal B-1a cells are long-lived cells, and have a renewal rate of only 1% per day (similar to conventional B cells). If our data are correct that B-1 cells account for such a high number of IgA-secreting cells in the gut lamina propria, the total number of (peritoneal) B-1 cells produced daily is clearly not enough to account for the large numbers of IgA precursor cells possibly needed every day. During their differentiation pathway to IgA-secreting cells, B-1 cells must thus divide several times after leaving the peritoneal cavity. Where this expansion (and isotype switching) occurs is currently not known.

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## CONCLUSIONS

The studies with B lineage chimeras and  $\mu, \kappa$  transgenic mice show that B-1 cells potentially can contribute significantly to the generation of IgA plasma cells in the murine intestine. Support for this hypothesis also comes from findings by others.<sup>3,24</sup> For example, several B-1 cell-lines (e.g. CH12) readily switch in vitro from IgM to IgA expression<sup>25</sup> and transplanting fetal omentum into severe combined immunodeficiency (SCID) mice not only results in the reconstitution of exclusively B-1 cells but also in the development of IgA plasma cells in the small intestine.<sup>26</sup> Finally, studies by Pecquet *et al.*<sup>27</sup> have recently shown that B-1 cells are responsible for a protective mucosal immune response to *Salmonella typhimurium*: only when repopulated with cell sources containing B-1 cells, CBA/N Xid mice (which are normally devoid of B-1 cells<sup>5</sup>) produce serum and mucosal IgA responses after oral immunization with *Salmonella*.

Although the data thus indicate that B-1 cells can generate significant numbers of IgA plasma cells, it is still not known what the relative contribution is of B-1 derived IgA cells in normal, untreated animals. It is also not known whether both B-1a and B-1b cells are involved in the mucosal humoral immune response and transfer studies with sorted B-1 subpopulations are required to resolve this point. The most intriguing questions that also remains to be answered are of course whether there are differences in the IgA repertoire derived from conventional B cells and of B-1 cells and whether the two IgA's exert different functions.

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