Letters Response

Tissue distribution of lymphocytes and plasma cells and the role of the gut: response to Pabst *et al.*

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We would like to thank Drs. Pabst, Russell and Brandtzaeg [1] for an extensive commentary of our recently published Opinion article on problems associated with the quantification of gut lymphocytes [2]. In their letter, the authors pointed out that the commonly used term 'lymphocyte' in a strict sense does not include plasma cells, even though lymphocytes and plasma cells are 'lymphoid cells'. Because many of the studies we cited quantified lamina propria (LP) lymphocytes, Pabst *et al.* [1] argued that, in contrast to our claim, it remains unknown whether spleen and lymph nodes are the major lymphoid compartment in humans if one also accounts for the distribution of plasma cells in the body.

Indeed, in many of the previous studies, lymphoid cells, isolated from lymph nodes, spleen, bone marrow, gut LP and gut epithelium were called lymphocytes. Unfortunately, most of these studies have not reported what cell types were included under the category of 'lymphocytes'. In several papers on gut immunology, the term lymphocyte is used interchangeably with lymphoid cells, and it is unclear whether or not previous studies that we cited included gut plasma cells in their estimate of the LP lymphocytes. Nevertheless, using the strict definition of lymphocyte of Pabst *et al.* [1] for previously published studies, our analysis does suggest that approximately 10% of the total body lymphocytes (senso stricto) reside in the gut LP, and this estimate is reasonably consistent for several mammalian species analyzed [2]. Additional studies are needed to confirm this result for all lymphoid cells (meaning lymphocytes and plasma cells).

Pabst *et al.* [1] argued that the distribution and the number of immunoglobulin (Ig)-secreting cells in humans is well understood. However, to the best of our knowledge, there is no single study that estimates both the number of Ig-secreting cells in the whole body and in the gut LP of humans, and only one such study exists in mice [3]. Moreover, there are some inconsistencies in estimating the number of lymphoid cells in the gut LP, both in humans and mice, casting doubt on whether the distribution of Ig-producing cells in these species is in fact well understood.

Estimation of the total number of Ig-secreting cells in the human gut has been done in a pioneering study by Brandtzaeg and Baklien [4]. Using histological samples, the authors defined a part of the gut LP as a 'mucosal unit' (Figure 1). The authors extensively examined the number of cells secreting IgA, IgG and IgM per mucosal unit in different parts of the human intestine [4]. A simple calculation suggests that the human small intestine contains at least 2×10^{10} of Ig-secreting cells (see legend of Figure 1). This is indeed a large number, because in another study, it was estimated that all other organs in humans, including spleen (4.7×10^8), lymph nodes (9.8×10^8) and bone marrow (2.3×10^{10}), contain almost 2.5×10^{10} Ig-secreting cells [5]. The estimate of 2×10^{10} plasma cells is consistent with another estimate of 3×10^{10} lymphoid cells (meaning lymphocytes and plasma cells) found in the gut LP [6]. This indeed would argue that the majority of lymphoid cells in the gut LP in humans are plasma cells. In mice, van der Heijden *et al.* [3] found that in 20-week-old C3H/He mice, the small intestine contains most of the Ig-secreting cells (1.58×10^7), followed by the spleen (1.27×10^6) and the bone marrow (5.2×10^5). These few studies indeed suggest that a large fraction (or even the majority) of Ig-secreting cells in humans and mice reside in the gut.

However, problems arise when we compare these estimates of the number of Ig-secreting cells with other estimates of the number of lymphoid cells in the gut LP. For example, by quantifying the number of lymphoid cells (meaning lymphocytes and plasma cells) per gram of the gut tissue in humans, it was also found that there are only $\sim 8.5 \times 10^9$ lymphoid cells per human gut [2]. Also, only 10^6–10^7 lymphoid cells (meaning lymphocytes and plasma cells) have been recovered from the murine small intestine in a series of studies [7–9], which is up to 10 times fewer cells than recovered in the study of van der Heijden *et al.* [3]. Finally, restricting ourselves to T lymphocytes, only 7×10^9 T lymphocytes reside in the gut LP, accounting only for a few percent of the total body T lymphocytes [2,10] (I. Sereti, pers. commun.).

Such different estimates of the total cell numbers obtained in different studies could be because of several reasons, one of which is the experimental technique used. It is possible that preparation of single cell suspensions from organs such as the small intestine may result in the death of a large fraction of lymphocytes depending, for example, on the specimen size and treatment [7]. A recent study suggested that one could grossly underestimate the number of activated T-cell receptor–transgenic T cells in the gut LP by preparing single cell suspensions compared with histological analysis [11]. Obtaining estimates for the total number of lymphocytes from a histological sample is also not trivial because of the complex structure of the small intestine [12]. Clearly more studies are necessary to address the question of reliable estimation of cell numbers in different organs, especially in the gut.

Since the publication of our Opinion article, we have approached several leading groups in the mucosal immunology field with the suggestion to readdress the issue of distribution and numbers of lymphocytes (and plasma

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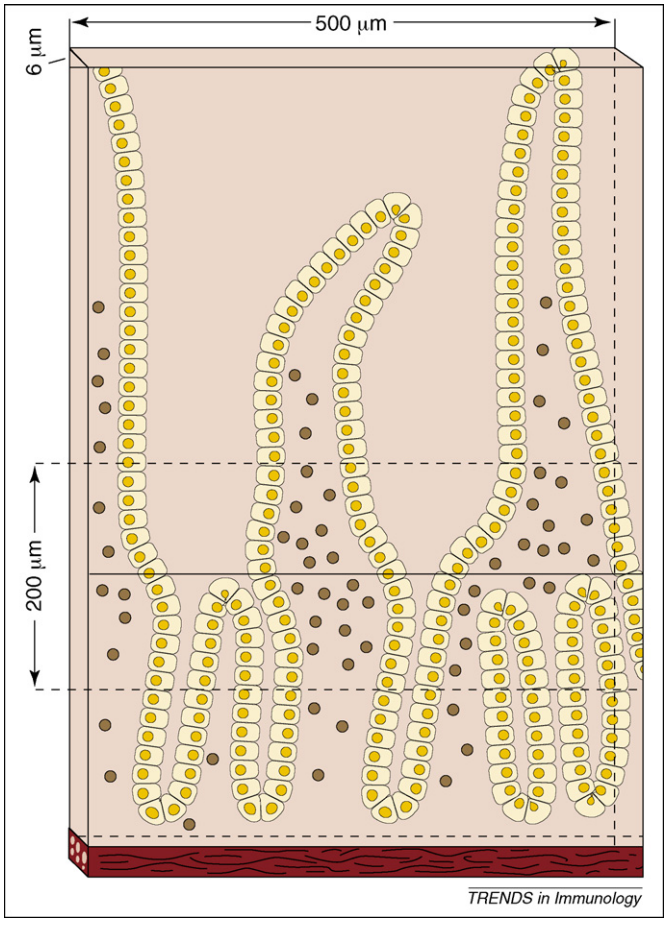

Figure 1. Section of lamina propria of the human intestine defined as a 'mucosal unit' [4]. A mucosal unit is a 6-μm section of the gut tissue with the length of 500 μm. On average, ~100 Ig-secreting cells (predominantly of the IgA isotype) are found in a mucosal unit of healthy humans (although, of course, this number varies in different parts of the intestine, [4]). Given this estimate, the total number of Ig-secreting cells per human small intestine can be calculated as follows. The inner diameter of the small intestine d is ~2–4 cm [12,13]. Taking 3 cm as an average, the circumference is $\pi d \approx 9.5$ cm. Given the thickness of the mucosal unit of 6 μm, the number of Ig-secreting cells per 500 μm of the gut is simply 100×9.5 cm/6 μm $\approx 1.6 \times 10^6$ cells. Given that the length of the small intestine is ~6 m [14], the total number of Ig-secreting cells in the small intestine is $1.6 \times 10^6 \times 6$ m/500 μm $\approx 2 \times 10^{10}$ cells. A similar (although slightly larger) number was reported in a previous study [4], even though that study did not describe how the calculation was made. The number of Ig-secreting cells may even be larger because of Kerckring folds in the small intestine and because of plasma cells residing in the large intestine. The cartoon is adapted with permission from Brandtzaeg and Baklien [4].

cells) in different organs of mice. Sadly, although the necessity of such studies is realized, leading scientists expect little success in getting funding for such basic research. Granting agencies and referees should become aware of our very limited knowledge of the distribution, number and turnover of lymphocytes in mammals and allow for more basic research addressing these fundamental questions in the future.

References

1 Pabst, R. *et al.* (2008) Tissue distribution of lymphocytes and plasma cells and the role of the gut. *Trends Immunol* 29, 209–210
2 Ganusov, V.V. and De Boer, R.J. (2007) Do most lymphocytes in humans really reside in the gut? *Trends Immunol.* 28, 514–518
3 van der Heijden *et al.* (1987) Contribution of immunoglobulin-secreting cells in the murine small intestine to the total 'background' immunoglobulin production. *Immunology* 62, 551–555
4 Brandtzaeg, P. and Baklien, K. (1976) Immunohistochemical studies of the formation and epithelial transport of immunoglobulins in normal and diseased human intestinal mucosa. *Scand. J. Gastroenterol. Suppl.* 36, 1–45
5 Turesson, I. (1976) Distribution of immunoglobulin-containing cells in human bone marrow and lymphoid tissues. *Acta Med. Scand.* 199, 293–304
6 Trepel, F. (1974) Number and distribution of lymphocytes in man. A critical analysis. *Klin. Wschr.* 52, 511–515
7 Davies, M.D. and Parrott, D.M. (1981) Preparation and purification of lymphocytes from the epithelium and lamina propria of murine small intestine. *Gut* 22, 481–488
8 Masopust, D. *et al.* (2001) Preferential localization of effector memory cells in nonlymphoid tissue. *Science* 291, 2413–2417
9 Resendiz-Albor, A.A. *et al.* (2005) Striking phenotypic and functional differences in lamina propria lymphocytes from the large and small intestine of mice. *Life Sci.* 76, 2783–2803
10 Ciccone, E. *et al.* (2007) CD4+ resoration in GALT may occur with prolonged HAART. In *Keystone Symposia on Molecular and Cellular Determinants of HIV Pathogenesis* (Malim, M.H. *et al.*, eds), Whistler
11 Honjo, K. *et al.* (2004) Activation and migration of allo-peptide specific TCR transgenic T cells in cardiac allograft rejection. *Cell Immunol.* 230, 44–55
12 Wilson, T.H. (1962) *Intestinal Absorption,* W. B. Saunders
13 Snyder, W.S. (1975) *Report of the Task Group on Reference Man,* Pergamon Press
14 Weaver, L.T. *et al.* (1991) Small intestinal length: a factor essential for gut adaptation. *Gut* 32, 1321–1323

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