staining artifacts, we would have double-stained for cytokeratin and AIRE as well. In addition, we detected similar cells expressing proinsulin, GAD and IA-2 in peripheral lymphoid tissues, which also expressed the transcripts encoding these molecules[5,8]. As the spleen and lymph nodes do not contain thymic epithelial cells, at least some of the bone marrow–derived APCs expressing peripheral proteins in lymphoid tissues should transcribe the corresponding genes. Accordingly, we detected insulin mRNA in proinsulin$^+$CD11c$^+$ DCs after the cells were sorted from human spleen and showed that similar cells expressing proinsulin existed in peripheral blood[9]. In contrast, Derbinski *et al*. did not detect insulin, GAD or IA-2 transcripts in RNA samples from splenic CD11c$^+$ DCs. This may reflect methodological differences, as we enriched our DC preparations by costaining for CD11c and proinsulin.

It is even more difficult to reconcile the findings of Derbinski *et al.*[1] with those of Throsby *et al*.[3]. Both groups studied the C57BL/6 mouse strain and used an essentially identical approach. This approach used RT-PCR to detect the expression of specific transcripts in cell populations that were purified by sorting after cells had been stained for cell surface markers specific for thymic epithelial cells, macrophages or DCs. Perhaps differences in tissue or cell manipulation and cell sorting could help explain conclusions that are so dramatically different? The variable amounts of RNA available for RT-PCR experiments from sorted cell populations that are unequally represented in the thymus, as reported by Derbinski *et al*.[1], may also be a factor, as thymic transcription of genes encoding peripheral proteins is extremely low[4].

In our opinion there is evidence that both bone marrow–derived APCs and thymic epithelial cells express peripheral proteins in the thymus, but it remains unclear whether the pattern of expression is complementary or somewhat redundant. In addition, the observation that at least some of these proteins are also expressed by bone marrow–derived APCs in peripheral lymphoid tissues in humans indicates that thymic epithelial cells cannot fully explain self-molecule expression by specialized APCs of the immune system.

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Response

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Pietropaolo *et al.* and Pugliese *et al*. are correct in arguing that our study does not entirely rule out the possibility that other thymic cells can express a diverse range of tissue-specific antigens. It was neither our intention, nor do we consider it appropriate, to address the issue of gene expression in a categorical yes-or-no fashion. It has long been known that a certain degree of so-called illegitimate transcription allows for the presence of one or few transcripts of any given gene in any given cell[1,2]. Consequently, the detection of a specific transcript reflects, to a large extent, the sensitivity of the method applied, and the designation of a positive signal has to be defined in the particular context. We compared expression of tissue-specific genes in five different thymic cell types by carefully calibrating the amount of input cDNA according to the expression levels of housekeeping genes. The PCR data thus allow for an estimate of the relative abundance of transcripts in the respective cell types. The central observation of our study is that mTECs are clearly distinct in the strength as well as the diversity of genes that are promiscuously expressed. We have, in the meantime, extended this observation to mTECs isolated from the human thymus (B. K. *et al.* unpublished data).

The failure of Pugliese *et al*.[3] to detect the expression of endocrine pancreas gene products (for example, insulin and GAD) in keratin-positive cells from the human thymus and yet their success in detecting these gene products in DCs and macrophages from the thymus and spleen is puzzling.

Pugliese *et al*. incorrectly state that we used an identical approach to Throsby *et al*.[4]. Throsby *et al.* analyzed sorted DCs and macrophages, but did not relate gene expression in these cell types to equally pure preparations of cTECs or mTECs. Instead, TEC-like cell lines and small numbers of cells of deoxyguanosine-treated thymic lobes were compared and found to be negative. We have confirmed the finding that established thymic epithelial cell lines do not display promiscuous gene expression (L. K., unpublished data). There may be numerous reasons for differences in gene expression between *ex vivo* isolated cells and long-term cell lines, including signals from the *in vivo* microenvironment. We analyzed freshly isolated cells without an intervening culture period[5].

Pietropaolo *et al*. allude to the ill-defined PAE cells as being "an additional cell type" (distinct from mTECs) that expresses peripheral antigens. We think it likely that PAE cells are, in fact, mTECs or a subset of mTECs. This is because of the observation that the tolerogenic competence of the PAE cells, as defined by Hanahan's group, could be transferred with the thymus into nude mice and the tolerance remained robust several months after reconstitution of the T cell compartment[6].

Thus, our study does not exclude expression of certain peripheral antigens in thymic cells of hematopoietic origin, indeed we have reported tolerance induction to proteolipid protein associated with bone marrow–derived cells[7]. In the case of genes of the endocrine pancreas, however, expression in thymic and peripheral DCs appears at least two orders of magnitude lower than in mTECs. At present we cannot reconcile our data with those reported in studies that failed to detect insulin- and GAD-specific mRNA or protein in mouse[4] and human mTEC[3].

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