Heat-Stable Antigen May Be an Early Marker of Extrathymic Murine Intestinal Intraepithelial Lymphocytes

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Using a system of bone marrow (BM) hematopoietic repopulation of irradiated euthymic and athymic mice, we have examined the early stages of extrathymic T-cell development within the murine small intestine epithelium. During a period of active extrathymic T-cell development, two distinct populations of intraepithelial lymphocytes (IEL) were present. One consisted of CD3+ lymphocytes with phenotypic properties of mature IEL. The other population consisted of a transient IEL subset that increased in abundance between days 5 and 14 post-BM transfer, and then declined. The majority of transient IEL in both types of mice expressed the heat-stable antigen, of which some cells coexpressed CD3 but were void of other markers common to mature T cells. Studies using freshly extracted IEL from normal nonirradiated mice found that ~3% to 5% of the IEL had phenotypic properties similar to the transient IEL observed during repopulation of radiation chimeras, indicating that such IEL are present within the gut epithelium of normal, nonirradiated mice. Identification of this IEL subset should greatly facilitate studies of extrathymic IEL development.

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STUDIES USING congenitally athymic nude mice and bone marrow (BM) radiation chimeras show that a large proportion of murine intestinal intraepithelial lymphocytes (IEL) develop extrathymically as shown by the presence of mature T cells in intestinal but not in extraintestinal peripheral compartments. Still other studies suggest that the gut itself is capable of regulating IEL differentiation and function. At present, however, little is known about specific lymphocyte subsets that demarcate stages of development along the IEL extrathymic pathway. Identification of such markers is critical for understanding the process of extrathymic IEL ontogeny. In previous studies, we have used an experimental system that permits us to track the kinetic repopulation of lymphoid cells in the gut, the thymus, and peripheral immune compartments following BM transfer into irradiated euthymic mice. That study found that, following BM transfer into lethally irradiated mice, T-cell repopulation of the intestine epithelium is initiated before that of the thymus and well before mature T cells are present in the spleen, thus providing a window from which to evaluate extrathymic IEL development in euthymic mice, and to compare that with what occurs in athymic chimeras. In the present study, we have identified an IEL subset that has phenotypic properties of immature T cells. Those IEL are present during an active phase of extrathymic IEL development in both euthymic and athymic chimeras and normally are present in IEL from adult mice.

MATERIALS AND METHODS

Mice. C57BL/6, BALB/c, and CB6F1 (BALB/c × C57BL/6)F1 mice were raised at the University of Tulsa from breeding stocks purchased from the Jackson Laboratories, Bar Harbor, ME.

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RESULTS

Identification of a transient IEL subset present during extrathymic lymphoid cell repopulation within the small intestine epithelium of lethally irradiated euthymic and athymic mice. Small intestine–extracted cells from euthymic and athymic mice were studied by flow cytometric (FCM) analyses between days 3 and 27 post-BM transfer according to cell size and granularity and for expression of lymphocyte markers. Consistent with published studies from this and other laboratories, the numbers of IEL declined to less than 10% of normal values by days 5 to 7 after irradiation (1.3 ± 0.4 × 106 cells/irradiated mouse compared to 11.7 ± 1.2 × 106 cells/unirradiated mouse). After day 7, numbers of IEL increased progressively. Based on physical criteria, four cellular populations were evident in FCM scattergrams of small-intestine preparations during the period of study (see Fig 1A, day 5). These consisted of: (1) epithelial cells and enterocytes, (2) a population of typical IEL as...
Fig 1. Cellular populations in small intestine epithelium-extracted preparations from (A) euthymic radiation chimeras and (B) athymic radiation chimeras between days 3 and 27 post-BM transfer. Four populations are evident consisting of enterocytes/epithelial cells, typical IEL, transient IEL, and cell debris. These findings were observed in three separate experiments for each type of mouse.

described elsewhere, a population of transient, slightly smaller lymphocytes, and (4) cellular debris. Greater than 95% of cells in the epithelial, typical IEL, and transient cell populations were viable based on trypan blue exclusion.

In euthymic chimeras, typical IEL were present throughout the period of study; however, there was a sharp reduction in proportion of those cells until day 9 after reconstitution, after which that subset increased proportionally (Fig 1A). Conversely, the transient IEL subset was evident from day 5 to 14 post-BM transfer, comprising the dominant cell population by day 9. By day 11, typical IEL were again evident, and by day 27 they were the primary population of lymphoid cells in the small intestine epithelium.

In athymic chimeras, the overall pattern of cell repopulation was consistent with that of euthymic mice, although some differences existed. The transient IEL were clearly evident only during days 7 and 9 after irradiation (Fig 1B), a time-frame consistent with, although slightly shorter than that of euthymic mice. Typical IEL were evident in athymic chimeras throughout the time of study. To determine at what point donor-derived cells appeared among the IEL, cells were analyzed for H-2 expression. Similar to what has been previously reported from this laboratory, the majority of typical IEL from euthymic mice were donor-derived cells by day 7 post-BM transfer (data not shown). The majority of typical IEL were of donor origin by day 9 post-BM transfer (Fig 2, top), and transient IEL were of donor origin by day 7 post-BM transfer in athymic mice (Fig 2, bottom), thus showing that transient IEL were not residual host lymphocytes.

Transient IEL in euthymic and athymic mice express a phenotype of immature T cells. Typical and transient IEL
subsets in both euthymic and athymic chimeras were studied for expression of lymphocyte markers including three markers associated with BM hematopoietic stem cells and/or immature T cells: HSA, CD44 (Pgp-1), and Sca-1. Throughout the study, neither CD44 nor Sca-1 were expressed to any significant extent on either the typical or the transient IEL population (data not shown). From days 5 to 14, the majority of typical IEL were CD3+, HSA- cells although slightly more CD3+, HSA+ cells were present at day 9 post-BM transfer (Fig 3, top). Similar to what has been previously reported for those cells, TCR-αβ and TCR-γδ was expressed in about equivalent proportions on the typical IEL (data not shown). That pattern held true for typical IEL in athymic mice; however, a minor subset of CD3+, HSA+ cells also was evident at days 9 and 11 post-BM transfer (Fig 4, top).

Unlike the typical IEL, most transient IEL in euthymic and athymic chimeras were HSA+ cells, some of which coexpressed CD3, more so for athymic than euthymic chimeras (Figs 3 and 4, bottom). That pattern was consistent in several isolates and may reflect the fact that transient IEL in athymic mice were present during a shorter period of time than in euthymic mice, thus more transient cells in athymic mice appear to be in an intermediate stage. Transient IEL, including those that expressed CD3, did not express CD4 or CD8. Only about half of the CD3+ transient IEL expressed TCR-αβ or TCR-γδ (data not shown); the reason for differences in CD3 and TCR expression is not clear at this point. Virtually all transient IEL were Thy-1+ cells (Fig 5). Because transient IEL did not express surface immunoglobulin (Ig) (Fig 5), HSA+ transient cells were not B cells. Moreover, transient IEL were not erythrocytes because erythrocytes had been lysed before FCM analyses and were not evident microscopically after lysis. Nor were transient IEL granulocytes, monocytes, or mast cells, all of which are larger and substantially more granular than typical IEL, whereas transient IEL were always smaller than typical IEL. Thus, the transient IEL were phenotypically similar to early T-cell precursors present during intrathymic T-cell development, yet they were present in euthymic mice before T-cell repopulation of the thymus, and also were present in athymic chimeras.

Cells with properties of transient IEL are present among IEL from normal nonirradiated adult mice. Experiments were performed to determine whether cells with physical

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Fig 2. H-2k expression on CD45+ typical IEL (top) and transient IEL (bottom) in athymic radiation chimeras.

Fig 3. Two-color FCM analyses of CD3 and HSA expression on typical (top) and transient (bottom) IEL from euthymic radiation chimeras between days 5 and 14 post-BM transfer.

Fig 4. H-2k expression on CD45+ typical IEL (top) and transient IEL (bottom) in athymic radiation chimeras.
and phenotypic properties similar to those observed during extrathymic T-cell repopulation in the gut were present in IEL isolates from normal nonirradiated mice. Freshly extracted IEL were separated according to cell density using techniques used to study heterogeneous cell populations such as IEL. In discontinuous Percoll gradients consisting of 40%, 50%, 55%, and 70% Percoll, three fractions (IEL-40, IEL-50, and IEL-55, respectively) were obtained. Shown in Fig 6, the IEL-40 fraction consisted of a single lymphocyte subset, the IEL-50 cells contained two distinct subsets consisting of typical IEL and a minor subset of slightly smaller cells, and the IEL-55 fraction consisted of a single subset. Approximately 90% of the total IEL consisted of IEL-40 and IEL-50 fractions (larger population). About half to three fourths of the IEL-40 and IEL-50 fractions expressed Thy-1 (Fig 6); none expressed surface Ig (Fig 6). The IEL-40 and IEL-50 (larger population) were CD3+ , HSA-, whereas most IEL-50 cells (smaller population) were CD3+, HSA+, although HSA-, CD3+ cells and HSA+, CD3- cells also were present. IEL-55 cells consisted of three subsets that closely resembled the transient IEL observed in radiation chimeras. These included HSA+, CD3- cells; HSA+, CD3+ cells; and HSA-, CD3+ cells (Fig 6); those cells comprised about 10% of the total IEL. Those findings were observed in several IEL isolates.

**DISCUSSION**

Because the IEL are greatly enriched for extrathymic T cells, which appear to develop locally within the gut, it is feasible that the stages of differentiation in that process resemble what occurs during T-cell development within the thymus. However, it is surprising that of the many IEL studies performed to date, few have examined the expression of markers associated with developing T cells. The data presented here provide evidence for an IEL subset with characteristics of an immature T-cell population that is present during an active phase of extrathymic IEL development.

Although we cannot totally rule out the possibility that in adult mice the HSA+, CD3- cells were immature thymocytes that had migrated to the gut, current evidence suggests that such does not occur in mice. Whereas in chickens, some embryonic thymocytes may migrate to the small intestine.
murine thymocytes do not appear to repopulate the gut. We also have found no evidence for trafficking of thymocytes to the gut in either normal or irradiated mice (M. Hamad and J.R. Klein, unpublished observation). Those differences may reflect biologic variations between species. The possibility that the transient IEL were of thymus origin is rendered further unlikely by the finding that HSA+ cells were present in abundance in the gut of euthymic chimeras between days 5 and 14 post-BM transfer, at a time when the thymus is nearly void of lymphoid cells, and because transient IEL were present during that time in athymic chimeras. Moreover, HSA+ IEL have been noted in previous IEL studies using congenitally-athymic nude mice and athymic chimeras. In the latter, both HSA+, CD3+, and HSA+, CD3+ populations were present. Thus, HSA+ IEL are not restricted to a particular experimental system. Rather, cells with characteristics of transient IEL appear to be a natural component of the IEL in adult, nonirradiated mice, albeit in low numbers (~3% to 5% of total IEL, as calculated from the distribution of cell in Fig 6).

Finally, this study delineates a pathway of differentiation of IEL in normal adult mice that is readily amenable to detailed study in the context of extrathymic IEL development. The IEL-55 cell fraction in normal mice appears to be enriched for precursor cells destined to become mature IEL, ie, IEL-50 and IEL-40. The capacity to readily separate those populations by cell density will greatly facilitate detailed studies into mechanisms of IEL extrathymic development.

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DG Stickney, J Wang, M Hamad and JR Klein