From the Cover: Functional CD8+ but not CD4+ T cell responses develop independent of thymic epithelial MHC

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Functional CD8\(^{+}\) but not CD4\(^{+}\) T cell responses develop independent of thymic epithelial MHC

Marianne M. Martinic*†, Maries F. van den Broek*, Thomas Rülicke§, Christoph Huber†, Bernhard Odermatt**, Walter Reith††, Edit Horvath*, Raphael Zellweger*, Katja Fink*, Mike Recher*, Bruno Eschli*, Hans Hengartner*, and Rolf M. Zinkernagel*‡

*Institute of Experimental Immunology, †Institute of Laboratory Animal Science, and **Institute of Clinical Pathology, University Hospital Zurich, CH-8091 Zurich, Switzerland; ‡Department of Immunology, The Scripps Research Institute, La Jolla, CA 92037; and ††Department of Pathology and Immunology, University of Geneva Medical School, CH-1211 Geneva 4, Switzerland

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The role of nonthymic epithelial (non-TE) MHC in T cell repertoire selection remains controversial. To analyze the relative roles of thymic epithelial (TE) and non-TE MHC in T cell repertoire selection, we have generated tetraparental aggregation chimeras (B6-nude\(\times\)BALB/c and B6\(\times\)BALB/c-nude) harboring T and B cells from both parents, whereas TE cells originated exclusively from the non-nude donor. These chimeras mounted protective virus-specific TE and non-TE MHC-restricted T cell responses. To further evaluate whether non-TE MHC alone was sufficient to generate a functional T cell repertoire, we generated tetraparental aggregation chimeras lacking MHC class II (B6-nude\(\times\)MHCII\(^{7}\)) or both MHC molecules (B6-nude\(\times\)MHCII\(^{-/-}\)II\(^{-/-}\)) on TE cells, but not on cells of B6-nude origin. Chimeras with MHC-deficient TE cells mounted functional virus-specific CD8\(^{+}\) but not CD4\(^{+}\) T cell responses. Thus, maturation of functional CD4\(^{+}\) T cell responses required MHC class II on thymic epithelium, whereas CD8\(^{+}\) T cells matured in the absence of TE MHC.

T cell selection | tetraparental aggregation chimeras

The thymus is the organ responsible for the differentiation of progenitor T cells into mature T cells expressing self-MHC-restricted and self-tolerant T cell receptors (TCR) (reviewed in refs. 1 and 2). It is well established that thymic epithelial (TE) cells play a very important role in T cell repertoire selection (ref. 3; reviewed in refs. 4 and 5). However, the role for non-TE cells in T cell repertoire selection is uncertain. Most of the studies investigating non-TE MHC-associated T cell repertoire selection came to the conclusion that non-TE MHC is not efficient in selecting a functional T cell repertoire (ref. 6; reviewed in refs. 4 and 7). The few studies showing involvement of non-TE MHC in T cell repertoire selection were not accepted for several reasons: because non-TE MHC-restricted T cell responses were weak and required several rounds of restimulation, because the antigens used were not defined at the peptide level ([Tyr,Glu]poly(DL-Ala)poly(Lys)-polymer], or because the results were solely from in vitro experiments (8–17). Although studies with nude mice reconstituted with a fully allogeneic thymus graft showed almost exclusive restriction to nude MHC alone, these studies were also not accepted because of suspected rescue of nude thymic rudiment (18). More recently, however, the availability of new tools and mice has allowed the controversial issue of the role of non-TE versus TE MHC in T cell repertoire selection to be readdressed. We have generated tetraparental aggregation chimeras from thymus-deficient nude mice and T and B cell-deficient Rag\(^{-/-}\) mice (19). In these Rag\(^{-/-}\)benudef\(^{4}\) tetraparental aggregation chimeras TE cells were exclusively of Rag\(^{-/-}\) H-2\(^{b}\) origin; however, T and B cells were exclusively of nude H-2\(^{a}\) origin. After infection of these chimeras with lymphocytic choriomeningitis virus (LCMV) or vesicular stomatitis virus (VSV), the MHC restriction and functionality of T cells were analyzed. Protective LCMV-specific CD8\(^{+}\) T cell responses restricted to TE and non-TE MHC were detected in all chimeras tested. In addition, CD4\(^{+}\) T cells restricted to non-TE MHC were present and functional as shown by the presence of high titers of CD4\(^{+}\) T cell-dependent VSV-specific neutralizing antibodies comparable to those present in control mice. However, concerns emerged that T cells restricted to nude MHC in these chimeras might have had a survival advantage in the periphery over TE MHC-restricted T cells. It is well known that after leaving the thymus mature T cells are able to survive only in the periphery when their TCR remain in continuous interaction with TCR-restricting MHC molecules (20–22). In the periphery, mature T cells will primarily be in contact with their surrounding T and B cells, which in the Rag\(^{-/-}\)benudef\(^{4}\) chimeras exclusively express nude MHC. Even if the nude MHC-restricted T cells after leaving the thymus were initially a minor population, in the periphery these T cells might have a survival advantage over the initially more numerous TE MHC-restricted T cells.

Therefore, to ensure that neither TE MHC- nor nude MHC-restricted T cells have a survival advantage in the periphery, we have now generated B6-nude\(\times\)BALB/c and B6\(\times\)BALB/c-nude tetraparental aggregation chimeras. In these chimeras, TE cells are exclusively of BALB/c or B6 origin, respectively, T and B lymphocytes and professional antigen-presenting cells (APC), however, originate from both donors. We infected this set of chimeras and control mice with LCMV and analyzed MHC restriction and function of both CD4\(^{+}\) and CD8\(^{+}\) T cells. Our results also show that T cells from B6-nude\(\times\)BALB/c and B6\(\times\)BALB/c-nude chimeras were restricted to TE and non-TE MHC. CD4\(^{+}\) and CD8\(^{+}\) T cells were both functional, and chimeras showed protective immune responses comparable to those from control mice.

As already mentioned above, TE cells are involved in the selection of a functional T cell repertoire. However, it is unclear whether the presence of MHC molecules on these TE cells is required for T cell repertoire selection or whether MHC molecules expressed by non-TE cells are sufficient to select a functional T cell repertoire. Previous studies with bone marrow chimeras had shown that non-TE MHC inefficiently selects a CD8\(^{+}\) T cell repertoire (12, 23). Furthermore, experiments using bone marrow chimeras with hosts harboring MHC class II-deficient TE cells showed that CD4\(^{+}\) T cells were not positively selected (refs. 24 and 25; reviewed in ref. 26). In contrast, in C2ta pIV\(^{-/-}\) mice, which lack MHC class II...
expression on TE cells but not on professional APC and which have MHC class II-sufficient non-TE cells in the thymus, low levels of CD4+ T-cell-deficient NP-CGG-specific IgG were detected (27). We analyzed whether, in a thymus with MHC-deficient TE cells but MHC-sufficient non-TE cells present from birth, the selected CD4+ and CD8+ T cells were able to mount functional virus-specific T cell responses. To analyze these questions, we generated a second set of tetraparental aggregation chimeras and used C2a pLV+/+ and C2a pLV−/− mice as control mice. We first generated B6-nude→MHCII−/−/−/− tetraparental aggregation chimeras. In these chimeras, TE cells are exclusively of MHCII−/−/−/− origin and therefore lack MHC class I and II molecules. All other cells including professional APC originate from both donors, but only those from nude origin will express MHC molecules. Next we generated B6-nude→MHCII−/−/−/− tetraparental aggregation chimeras. In these chimeras, TE cells originate exclusively from MHCII−/−/−/− mice and, therefore, lack MHC class II but not MHC class I molecules. Again, all other cells originate from both donors; therefore, professional APC will express MHC class II molecules only when they are derived from nude origin. We infected these chimeras and control mice with LCMV and VSV and analyzed the function of both CD4+ and CD8+ T cells. CD8+ T cells from B6-nude→MHCII−/−/−/− chimeras but not from MHCII−/−/−/− controls showed virus-specific immune responses. Virus-specific CD4+ T cells, however, could not be detected in mice expressing MHC class II-deficient TE cells.

Results

Functional CD8+ T Cell Responses in B6-nude→BALB/c and B6→BALB/c-c Nude Tetraparental Aggregation Chimeras Are Restricted to TE and Non-TE MHC. We started with the generation of B6-nude→BALB/c and B6→BALB/c-c nude tetraparental aggregation chimeras. In these chimeras TE cells were exclusively of BALB/c or B6 origin, respectively; however, all other cells, including lymphocytes and professional APC, were derived from both donors. We infected both chimeras and control mice i.v. with 200 pfu of LCMV strain WE (LCMV-WE). Seven days after infection, blood cells from B6-nude→BALB/c and B6→BALB/c-c nude chimeras were analyzed for chimerism by staining T cells, B cells, and macrophages for the expression of H-2b and BALB/c H-2d MHC class I molecules (Fig. 4A), which is published as supporting information on the PNAS web site). All cell types analyzed always showed two distinct populations, one expressing H-2b and the other expressing H-2d MHC class I molecules, confirming that indeed all lymphocytes, macrophages (Fig. 4A), and dendritic cells (data not shown) originated from both donors. Similar results were obtained by analyzing chimeras before LCMV infection and analyzing different tissues such as thymus, spleen, and mesenteric and inguinal lymph nodes (data not shown). These data indicated that, in the periphery of these chimeras, as opposed to the previous Rag−/−x nude d chimeras, neither non-TE MHC-restricted nor TE MHC-restricted T cells had a survival advantage. To confirm that TE cells in B6-nude→BALB/c and B6→BALB/c-c nude chimeras were exclusively of BALB/c or B6 origin, respectively, we stained thymic sections for expression of cytokeratin (universal marker for epithelial cells) and B6 H-2b or BALB/c H-2d MHC class II molecules using fluorescence microscopy (Fig. 4B). In B6→BALB/c-c nude chimeras, B6 H-2b but not BALB/c H-2d MHC class II staining colocalized with cytokeratin staining, which also coincided with the TE network, confirming that TE cells were exclusively of B6 origin (Fig. 4B, orange staining). H-2d MHC class II cytokeratin− cells were also present (Fig. 4B, bright green-yellow staining), confirming the existence of professional APC of BALB/c-c nude origin in the thymus; corresponding results were obtained with B6-nude→BALB/c chimeras (Fig. 4B).

To assess MHC restriction and the function of CD8+ T cell responses in these chimeras, 8 days after infection blood cells were stained with anti-CD8 and LCMV-specific tetramers (Fig. 4I).

Chimeric CD4+ T Cells Are Restricted to TE and Non-TE MHC and Provide Help to B Cells. We then analyzed MHC restriction and functionality of CD4+ T cell responses. For this purpose, chimeras and control mice were infected i.v. with 200 pfu of LCMV-WE. Because LCMV peptides binding to H-2Aa MHC class II molecules have not yet been identified, we restimulated chimeric and control splenocytes 8 days after infection with the LCMV-gp33 peptide (gp33) or LCMV-np118-126 (np118) peptide, and CD8+ T cells were analyzed for IFN-γ secretion (Fig. 1B). Again, CD8+ T cells from chimeric but not from control mice produced IFN-γ after restimulation with either peptide, indicating that both TE and non-TE MHC-restricted CD8+ chimeric T cells were functional (Fig. 1B). In addition, chimeric and control splenocytes from day-13 immune mice were restimulated for 5 days with LCMV peptide-labeled irradiated splenocytes and tested for their ability to lyse LCMV peptide-labeled target cells in a 5-h 3H-release assay (Fig. 1C). Splenocytes from chimeras lysed peptide-labeled target cells of both haplotypes, confirming the TE and non-TE MHC-restricted specificity of CD8+ chimeric T cells in an additional functional assay (Fig. 1C). Finally, in all chimeras tested LCMV was cleared by day 13 from blood, spleen, kidney, liver, and lung as efficiently as in control mice (data not shown).

In conclusion, even when lymphocytes from both parents were present, chimeric CD8+ T cells were restricted to TE and non-TE MHC, and, furthermore, they were functional and protective. However, it is noteworthy to mention that, in all chimeras tested, T cell responses restricted to TE MHC were stronger than the ones restricted to non-TE MHC (Fig. 1A–C). This outcome is probably because of the fact that TE MHC-restricted T cells were more numerous because of their survival advantage in the thymus due to higher cell numbers expressing TE than non-TE MHC.

Mice Lacking MHC Expression on TE Cells Still Have Functional CD8+ T Cell Responses. To analyze whether non-TE MHC alone was sufficient in selecting a functional T cell repertoire or whether and which MHC molecules were needed on TE cells for proper T cell
repertoire selection, we generated tetraparental aggregation chimeras from thymus-deficient B6-nude mice and MHC class I and class II double-deficient mice. In the resulting B6-nude→MHCI−/−II−/− tetraparental aggregation chimeras, TE cells and all other cells of MHCI−/−II−/− origin were MHCI-deficient, whereas cells of B6-nude origin were MHC-sufficient. Immunofluorescence analysis of thymic sections confirmed the absence of MHC molecules on TE cells and the presence of MHC molecules on non-TE cells of B6-nude origin (Fig. 5, which is published as supporting information on the PNAS web site). To analyze whether selection on non-TE MHC alone was sufficient to generate a repertoire that can give rise to a functional CD8+ T cell response, chimeric and control mice were infected with 200 pfu of LCMV-WE i.v. Eight days after infection, blood and CD8+ T cells were stained for binding to the different LCMV-specific tetramers, gp33/D6 and np396/D6 tetramers (Fig. 2A). CD8+ T cells from chimeras and B6 mice always showed binding to either gp33/D6 or np396/D6 tetramers (Fig. 2A Top and Middle). However, MHCI−/−II−/− mice practically were devoid of CD8+ T cells, and the few remaining CD8+ T cells did not bind to any of the tetramers (Fig. 2A Bottom). Similar results were obtained after analysis of splenic CD8+ T cells (data not shown). These results indicated that mice lacking MHC expression on TE cells but expressing MHC molecules on all other TE cells, both in the thymus and in the periphery showed selection and survival of non-TE MHC-restricted CD8+ T cells able to bind LCMV-specific tetramers.

Next, day-8 LCMV-immune spleen cells from chimeras and control mice were restimulated for 6 h with LCMV-gp33–41 peptide (gp33) or LCMV-np396–41 peptide (np396), and CD8+ T cells were analyzed for IFN-γ secretion (Fig. 2B). Again, CD8+ T cells from B6 mice (Fig. 2B Top) and chimeras (Fig. 2B Middle) but not from MHCI−/−II−/− mice (Fig. 2B Bottom) produced IFN-γ after restimulation with either peptide, indicating that CD8+ T cells from chimeras but not from MHCI−/−II−/− mice were functional. Furthermore, the percentage of total CD8+ T cells in chimeras and in MHCI−/−II−/− mice compared with B6 mice was reduced by only 16 times in the chimeras, whereas it was reduced up to 25 times in MHCI−/−II−/− mice (Fig. 2B).

Thirteen days after infection, chimeric and control splenocytes were restimulated for 5 days with LCMV peptide-labeled spleen cells and assayed in a 5-h 51Cr-release assay for recognition of peptide-loaded EL-4 (H-2d) and P815 (H-2k) target cells. Curves represent individual mice. Data are representative of three independent experiments containing two to four mice per group. gp33, gp(33–41); np118, np(118–126). (B) Eight days after infection, splenocytes were restimulated in vitro for 6 h with LCMV-gp33–41 peptide (gp34) followed by surface staining for CD4 and intracellular staining for IFN-γ. Numbers beside circles indicate the percentage of CD4+ T cells secreting IFN-γ. Data are representative of four independent experiments and show one representative mouse per group (total, n = 5–6 mice per group). (C) Thirty days after infection, splenocytes were restimulated in vitro for 5 days with LCMV peptide-labeled spleen cells and assayed in a 5-h 51Cr-release assay for recognition of peptide-loaded EL-4 (H-2d) and P815 (H-2k) target cells. Curves represent individual mice. Data are representative of three independent experiments containing two to four mice per group. gp33, gp(33–41); np118, np(118–126). (D) Eight days after infection, splenocytes were restimulated in vitro for 6 h with LCMV-gp33–41 peptide (gp34) followed by surface staining for CD4 and intracellular staining for IFN-γ. Numbers beside circles indicate the percentage of CD4+ T cells secreting IFN-γ. Data are representative of four independent experiments and show one representative mouse per group (total, n = 5–6 mice per group).
Eight days after infection, splenocytes were restimulated in vitro with LCMV-gp64–80 peptide followed by surface staining for CD8 and intracellular staining for IFN-γ. Numbers in bold indicate the percentage of CD8+ T cells secreting IFN-γ, and numbers in italics indicate the percentage of total CD8+ T cells. Data are of one representative mouse per group (total, n = 3–4 mice per group). (C) Thirteen days after infection, splenocytes were restimulated in vitro for 5 days with LCMV peptide-labeled spleen cells and assayed in a 5-h 51Cr-release assay for lysis of peptide-loaded EL-4 (H-2b) target cells. Curves represent individual mice. Data are of one representative mouse per group (total, n = 2–3 mice per group). gp33, gp(33–41); np396, np(396–404).

Fig. 3. Mice lacking MHC class II expression on TE cells lack functional virus-specific CD4+ T cell responses. (A) Chimeras and control mice were infected i.v. with 200 pfu of LCMV-WE. Eight days after infection, splenocytes were restimulated in vitro for 6 h with LCMV-gp64–80 peptide followed by surface staining for CD4 and intracellular staining for IFN-γ. Top) Mice with MHC class II-sufficient TE cells (MHC II+ TE cells) and MHC class II-professional APC (MHCII+ prof. APC). Middle) Mice with MHC class II-deficient TE cells (MHC II− TE cells) and MHC II+ professional APC. Bottom) Mice with MHC II− TE cells and MHC II− professional APC. Numbers in bold indicate the percentage of CD4+ T cells secreting IFN-γ, and numbers in italics indicate the percentage of total CD4+ T cells. Data are of one representative mouse per group (total, n = 2–4 mice per group). (B) CD4+ T cell-dependent VSV-neutralizing IgG titers were monitored at different time points after i.v. infection with 2 × 106 pfu of UV-inactivated VSV-Indiana. Data represent mean ± SEM of three individual mice per group and are representative of one experiment.

APC of B6-nude origin (Fig. 6, which is published as supporting information on the PNAS web site, and data not shown). We also analyzed C2ta pIV−/− mice, which lack MHC class II expression on TE cells and on cells of nonhematopoietic origin after IFN-γ stimulation, whereas professional APC are MHC class II-sufficient (27) (Fig. 6). Wadlhuber et al. (27) had previously shown that CD4+ T cell responses against NP-CGG were greatly reduced in C2ta pIV−/− mice. We were interested to analyze the virus-specific CD4+ T cell responses in B6-nude→MHCII−/− II−/−, B6-nude→MHCII−/−, C2ta pIV−/−, and control mice after infection with 200 pfu of LCMV-WE. Eight days after infection, splenocytes were restimulated for 6 h with the LCMV-gp64–80 peptide, and CD4+ T cells were assayed for IFN-γ secretion (Fig. 3B). Mice with MHC class II-sufficient TE cells (B6 and C2ta pIV−/− mice) had a normal percentage of total CD4+ T cells, and LCMV-specific CD4+ T cells from these mice showed secretion of IFN-γ after restimulation with gp64 (Fig. 3B Top). In contrast, all mice with MHC class II-deficient TE cells had a low percentage of total CD4+ T cells, and, with the exception of the C2ta pIV−/− mice, none of them showed IFN-γ-secreting CD4+ T cell responses (Fig. 3B Middle and Bottom). Analysis of later time points after LCMV infection also yielded an absence of IFN-γ-secreting CD4+ T cell responses (data not shown). To investigate whether the relatively small population of CD4+ T cells could provide sufficient cognate help to B cells to enable them to produce virus-neutralizing antibodies, we infected B6-nude→MHCII−/− II−/−, B6-nude→MHCII−/−, C2ta pIV−/−, and control mice i.v. with 2 × 106 pfu of UV-inactivated VSV-Indiana (VSV-IND). It has been shown previously that VSV-neutralizing IgG responses are strictly dependent on CD4+ T cell help (30). Sera from VSV-infected mice
were analyzed at different time points for VSV-neutralizing IgG (Fig. 3B). Only mice with MHC class II-sufficient TE cells (B6 and C2a pIV/−/− mice) were able to mount high titers of VSV-neutralizing IgG (Fig. 3B). All mice with MHC class II-deficient TE cells, however, were unable to mount any VSV-neutralizing TE responses (Fig. 3B). The same was true for LCMV-specific CD4+ T cell-dependent IgG responses; none of the mice with MHC class II-deficient TE cells showed any LCMV-specific IgG titer (data not shown). Taken together, these results showed that selection of a functional CD4+ T cell repertoire required the presence of MHC class II-sufficient TE cells.

Discussion

The generation of tetraparental aggregation chimeras, B6-/nude→BALB/c and B6→BALB/c-nude, has enabled us to analyze the role of non-TE and TE MHC molecules in T cell repertoire selection in a model where lymphocytes originated from both donors but TE cells were derived exclusively from the non-nude donor. After LCMV infection, CD8+ and CD4+ T cells in these chimeras mounted functional and protective T cell responses comparable to control mice, and T cells were restricted to non-TE MHC. However, non-TE MHC-restricted T cell responses were somewhat lower than TE MHC-restricted responses. In our previously reported Rag-/-/b-nude and Scid-/-nude chimeras, primary CD8+ T cell responses restricted to non-TE MHC could always be measured in a direct ex vivo cytotoxicity assay, whereas in B6-nude→BALB/c and B6→BALB/c-nude chimeras only ∼25% of the mice showed primary CD8+ T cell responses restricted to non-TE MHC (data not shown); however, all responded well after secondary in vitro stimulation. This finding may reflect quantitative and/or qualitative differences favoring survival and/or proliferation of TE MHC-restricted T cells in the thymus, correlating with the smaller number of cells expressing non-TE MHC compared with the ones expressing TE MHC. In the periphery, both TE and non-TE MHC-restricted T cells should have a comparable survival-favoring environment because the proportion of lymphocytes, APC, and all other somatic cells originating from each donor was about equivalent. In our earlier series of Rag-/-/b-nude tetraparental aggregation chimeras, non-TE MHC-restricted T cells may have had a survival advantage because in the thymus as well as secondary lymphoid organs lymphocytes were exclusively of nude origin, and therefore the total number of cells expressing non-TE MHC was higher than the number of cells expressing TE MHC. The question of whether non-TE MHC alone is sufficient to efficiently select a functional T cell repertoire had been studied previously with bone marrow chimeras and TCR transgenic mice. Most of these studies came to the conclusion that non-TE MHC only inefficiently selected a T cell repertoire (12, 23). For example, Bix and Raulet (12) injected MHC class I-deficient bone marrow into irradiated MHC class I-deficient recipients and showed that CD8+ T cell numbers were reduced. Importantly, CD8+ T cells exhibited allospecific T cell responses only when primed and boosted several times, whereas usually these responses are very strong in the absence of priming and do not require extensive boosting. In our B6-nude→MHCII/−/−/I−/− chimeras, however, where TE cells are devoid of MHC molecules but non-TE cells are MHC-sufficient, CD8+ T cell numbers were reduced only 1.6 times. After LCMV infection, chimeras showed functional virus-specific T cell responses. It is important to remember that the chimeras described in this study, in comparison to bone marrow chimeras, were not lethally irradiated. Therefore, MHC-sufficient non-TE cells in our chimeras were present in the thymus from the beginning, probably giving the developing thymocytes restricted to non-TE MHC a survival advantage, which is unlikely to occur in bone marrow chimeras. In addition, irradiation damage and subclonal graft-versus-host or host-versus-graft reactions, which may additionally skew the outcome of the experiment, were circumvented by the present approach.

The detection of functional virus-specific CD8+ but not CD4+ T cell responses in mice with MHC-deficient TE cells was surprising. One possible explanation is that the total number of MHC class I and II molecules has to be above a certain threshold to ensure selection and survival of CD8+ and CD4+ T cells, respectively. Whereas TE cells are MHC-deficient in B6-nude→MHCII/−/− I−/− chimeras, all cells originating from nude donor express MHC class I molecules. Therefore, continuous interaction with MHC class I molecules is guaranteed both in the thymus and in secondary lymphoid organs. In this situation, CD8+ T cells with intermediate-to-high-avidity TCR seem to become positively selected and survive subsequently in the periphery. As already shown by previous studies, CD8+ T cells with low-avidity TCR probably will not be able to survive in these mice because of low total numbers of TCR-restricting MHC class I molecules (23, 31). In contrast, in mice with MHC class II-deficient TE cells, the total number of MHC class II molecules in the thymus, not on TE but on professional APC, is probably not sufficient to ensure selection and survival of CD4+ T cells.

Because thymocytes are in constant interaction with each other, one can speculate as to whether expression of MHC class II molecules on these cells would increase the total MHC class II numbers above the threshold level needed for CD4+ T cell selection and survival. In two recent studies this question was elegantly answered by the generation of transgenic mice expressing the human class II transactivator molecule (CIITA) under the control of the CD4 promoter or the proximal promoter of p56ck. The resulting CIITA+ mice expressed MHC class II molecules on immature thymocytes and mature T cells (32, 33). The authors generated irradiation bone marrow chimeras by injecting bone marrow cells from CIITA+ mice into irradiated MHC class II-deficient recipients and showed that successful CD4+ T cell selection occurred in the absence of MHC class II molecules on TE cells (32, 33). Whereas murine thymocytes do not express MHC class II molecules, human, guinea pig, rat, and sheep immature thymocytes and/or peripheral T cells express MHC class II molecules (32, 33) and could therefore participate in CD4+ T cell selection. Thus, the presence of any MHC class II (independent of haplotype or even species) on TE cells seems to be necessary and sufficient for the development of a functional CD4+ T cell repertoire, whereas MHC class II on non-TE cells alone was not sufficient, at least not in mice. This concept is supported by experiments in which mice received a xenogeneic porcine thymus graft. These mice showed the presence of CD4+ T cells restricted to porcine MHC class II even in the absence of porcine MHC class II in the periphery. The cells could survive in the periphery because of the presence of murine MHC class II Iα, which shows highly conserved amino acid sequence, with porcine MHC class II DQ probably substituting therefore the need for porcine MHC class II in the periphery (34). These results are compatible with an attractive generalization proposed here: Maturing T cells need to contact MHC but not a particular allelic form of MHC. MHC class II contact on TE or non-TE cells is sufficient for maturing CD8+ T cells; however, maturing CD4+ T cells require MHC class II contact on TE cells.

These findings suggest that positive selection reflects more the reaching of a quantitatively critical number of contacts between the TCR and its specific antigen presented on MHC class I or II (allele-independent) than of antigen-independent but MHC allel-dependent contacts (1, 2, 35–38).

Materials and Methods

Mice. Tetraparental aggregation chimeras were generated at the Institute of Laboratory Animal Science of the University of Zurich (Zurich, Switzerland), as previously described (19). C57BL/6 (H-2b) (B6) and BALB/c (H-2d) mice were purchased...
from Harlan (Horst, The Netherlands). C57BL/6-nude (B6-nude, H-2^d) and BALB/c-nude (H-2^k) mice were from Biological Research Laboratories (Fullsöldorf, Switzerland). MHCI^-/- II^-/- and MHCI^-/- II^-/- (H-2^d) mice were from Taconic Europe (Lille Skensved, Denmark). C2ta pIV^-/- and C2ta pIV^-/- mice (H-2^k) (27) were obtained from Walter Reith (University of Geneva Medical School, Geneva, Switzerland).

Mice were bred and maintained in specific pathogen-free conditions. Experiments were done according to institutional guidelines and Swiss federal and cantonal laws on animal protection.

Cell Lines, 51Cr-Release Assay, VSV-IND Neutralization Assay, and Virus. EL-4 (H-2^d) and P815 (H-2^k) cells were obtained from the American Type Culture Collection (Manassas, VA). The 51Cr-release assay after restimulation in vitro, LCMV-WE, and VSV-IND have been previously described (39-42).

Antibodies, LCMV Tetramers, and Peptides. All antibodies used for flow cytometry were obtained from BD Biosciences Pharmingen (San Jose, CA). Samples were acquired on a FACS Calibur and analyzed by using FlowJo software (Version 6.3.1; Tree Star, Ashland, OR). Double tetramer stains with phycoerythrin- and allophycocyanin-labeled tetramers have been described (19). The LCMV-specific peptides [gp33 (gp33–41, KAVYNFATC), gp64 (gp64–80, GDPYKGVVFQKSFVFD), np396 (np396–404, FQPQNGQFI), and np118 (np118–126, RPQASGVYM)] were synthesized by Neosystems (Strasbourg, France). Peptide stocks (10^-2 M) in DMSO were diluted 1/1,000 and 1/100,000 in medium for intracellular cytokine stains and for restimulations, respectively.

Intracellular Cytokine Staining for IFN-γ. Approximately 1 × 10^6 splenocytes were incubated for 6 h at 37°C in a total volume of 200 µl of medium with 2.5 µM relevant peptide, with medium alone, or with 50 ng/ml phorbol 12-myristate 13-acetate plus 500 ng/ml ionomycin, in the presence of 5 µg/ml brefeldin A and 50 units/ml recombinant mouse IL-2. After incubation, cells were surface-stained with anti-H-2D^d-FITC (clone 34-2-12) and anti-CD8b.2-phycocerythrin (clone 53-5.8). Cells were then fixed with 2% paraformaldehyde and permeabilized with permeabilization buffer (flow cytometry buffer containing 0.1% saponin) followed by intracellular staining with anti-mouse IFN-γ-allophycocyanin (clone XMG1.2).

Immunofluorescence. Immunofluorescence histology of TE cells has been previously described (19). Images were recorded with an Olympus BX61 fluorescence microscope (Olympus, Melville, NY) and processed by using AnalySIS Software (Soft Imaging System, Münster, Germany).

Statistical Analysis. Statistical analysis was performed with Prism 4 software (GraphPad, San Diego, CA).

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