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Impact of TCR Reactivity and HLA Phenotype on Naive CD8 T Cell Frequency in Humans

François Legoux,* Emilie Debeauquis,* Klara Echasserieu,* Henri De La Salle,† Xavier Saulquin,*† and Marc Bonneville*†

The impact of MHC phenotype on the shaping of the peripheral naive T cell repertoire in humans remains unknown. To address this, we compared the frequency and antigenic avidity of naive T cells specific for immunodominant self-, viral, and tumor Ags presented by a human MHC class I allele (HLA-A*02, referred to as A2) in individuals expressing or not this allele. Naive T cell frequencies varied from one Ag specificity to another but were restrained for a given specificity. Although A2-restricted T cells showed similar repertoire features and antigenic avidities in A2+ and A2− donors, A2 expression had either a positive, neutral, or negative impact on the frequency of A2-restricted naive CD8 T cells, depending on their fine specificity. We also identified in all donors CD4 T cells specific for A2/peptide complexes, whose frequencies were not affected by MHC class I expression, but nevertheless correlated with those of their naive CD8 T cell counterparts. Therefore, both selection by self-MHC and inherent TCR reactivity regulate the frequency of human naive T cell precursors. Moreover this study also suggests that T cell repertoire shaping by a given self-MHC allele is dispensable for generation of immunodominant T cell responses restricted by this particular allele. The Journal of Immunology, 2010, 184: 000–000.

The 10^15 TCRs that can be theoretically generated through somatic recombination of TCR V(D)J segment is well above the estimated 25–100 × 10^6 distinct TCRs that actually make up the peripheral T cell repertoire in a given individual (1). This severe attrition of peripheral TCR diversity is due, at least in part, to both thymic and peripheral selection processes. In particular, T cell precursors undergo both “positive” and “negative” intrathymic selection processes that allow generation of a functional yet self-tolerant T cell repertoire. Although “positive selection” favors maturation of developing T cells with intermediate avidity for self-peptide/MHC (pMHC), “negative selection” deletes or inactivates strongly self-reactive thymocytes (2–4). How precisely the peripheral T cell repertoire is shaped by positive and negative selection remains debated (5–7). Consistent with the view that positive selection biases the peripheral repertoire toward recognition of pMHC complexes related to those encountered in the thymus, T cells expressing a given transgenic TCR are selected in vitro and in vivo by a single or a restricted set of pMHC complexes only (8–10). Along this line, a single pMHC complex can positively select a diverse CD8 T cell repertoire, which is primarily restricted by the selecting H-2 allele (11). These results suggest a dominant role played by positive selection in imprinting preferential Ag recognition in the context of self-MHC alleles, a phenomenon also referred to as “self-MHC restriction.” However, seemingly opposite conclusions were previously drawn from analysis of CD4 T cells selected by a single pMHC class II complex. In the latter case, the diverse CD4 repertoire selected by the transgenic pMHC complex was in large part deleted, owing to its extensive cross-reactivity against self- and allogeic pMHC, consistent with a major role played by negative selection in shaping the T cell repertoire (12–14). Besides thymic selection, peripheral homeostatic processes, which favor survival or expansion of naive T cells able to establish continuous low-affinity interactions with self-MHC, could contribute to self-MHC focusing and repertoire remodeling as well (15, 16).

The paucity of naive T cells specific for a given Ag has hampered assessment of the overall impact of thymic selection and MHC background on the MHC restriction of peripheral T cells in nongenetically manipulated outbred individuals. Ex vivo analysis of the naive-specific T cell repertoire in a nontransgenic setting has recently been made possible, thanks to novel approaches based on either immunomagnetic enrichment for specific T cells using soluble fluorescent multimers of defined pMHC complexes (17–20) or use of libraries of amplified naive T cells (21). One of such studies suggested that expression of a given murine MHC class II allele had a limited impact on the frequency of naive T cells restricted by this allele (22). However, it strongly affected the T cell repertoire quality, mainly through negative selection of self-reactive clones. Whether or not similar observations apply to T cells from other species or restricted by other MHC classes remains unknown.

In this study, we exploited a pMHC multimer-based sorting approach to analyze the impact of expression of a given HLA class I allele on the frequency and quality of peripheral blood naive CD8 T cells directed against several A2 restricted by this particular HLA allele. Our results indicate that expression of a defined HLA allele has a predominantly positive, although heterogeneous, impact on the frequency of naive CD8 T cells restricted by this allele, depending on the peptide Ag studied. Moreover, we provide evidence that
naive CD8 T cell precursor frequency is regulated by the cross-reactivity level for the corresponding Ags of T cells selected by weakly or unrelated pMHC complexes.

**Materials and Methods**

**Antibodies**

PerCP-Cy5.5-conjugated anti-CD14, CD16, and CD19 (BD Biosciences, San Jose, CA); Amcyan-conjugated anti-CD1 (BD Biosciences); Pacific Blue-conjugated anti-CD8; allophycocyanin- or FITC-conjugated anti-CD4; APC-H7-conjugated anti-CD27; FITC-conjugated anti-CD11a; PE-Cy7-conjugated anti-CD5RO; allophycocyanin-conjugated anti-IFN-γ; PE-conjugated anti-TNF-α; FITC-conjugated anti-CD107; FITC-conjugated anti-IL-2; and FITC-conjugated anti-BV17 Abs were purchased from BD Biosciences. Anti-AV2 Ab was provided by D. Speiser (Ludwig Institute for Cancer Research, Lausanne, Switzerland) and conjugated to AlexaFluor 647 using a labeling kit (Molecular Probes, Eugene, OR).

**Peptides and pMHC multimers**

The following HLA-A*0201–restricted peptides were purchased from Proimmune: MelA27 (melanoma Ag, ELAGIGILTV), PGT178 (human PG transporter, LLAGIGTVPI), NS3 1073 (hepatitis C virus [HCV], CINGVWCITV), pp65 295 (human CMV [HCMV], GILGFVFTL), Gag 77 (HIV-1, SLYNTVATL), MPA 58 (human influenza virus, GILGFVFTL), p800 (MelA27 variant, ELAGWGLTV), and p21 (NS3 1073 variant, CIGVGCWTV). Soluble pMHC monomers were synthesized as described previously (23). HLA-A*0201 H chains used in this study carried mutations in the α3 domain that either reduce (A245V, referred to as multimers) (23, 24) or abrogate (D227K and T228A, referred to as CD8-null multimers) (25, 26) CD8 binding to MHCI class I. As previously described, the 245V mutants bind to both low- and high-affinity T cell clones irrespective of their valency but show decreased background binding to irrelevant CD8 T cells at high valencies (unlike wild-type pMHC multimers) (23). By contrast, the dual mutants pMHC fluorescent multimers bind to high-affinity T cells (i.e., expressing CD8-independent TCRs) only at low valencies but stain both high- and low-affinity T cells at high valencies (24, 27). In third, the high-affinity pMHC oligomers of low valencies multimerized with either PE- or allophycocyanin-labeled streptavidin (Prozyme [Hayward, CA] and Invitrogen [Carlsbad, CA], respectively) at a molar ratio of 4:1.

**Donor samples**

Cytapheresis samples were obtained from donors seronegative for HCMV, HCV, and HIV and presumably not at risk for infection. Blood samples from TAP-deficient donors were also included in this study. HLA class I genotyping was performed by Etablissement Français du Sang (Nantes, France).

**Multimer-based enrichment protocol**

PBMCs were obtained by Ficoll density gradient centrifugation (LSM, Eurobio, Les Ulis, France). A total of 10^7 freshly isolated PBMCs were incubated with 200 μl PE-conjugated multimers (10 μg/ml) for 30 min at room temperature and then washed with 15 ml ice-cold sorter buffer (PBS plus 0.5% BSA plus 0.2% EDTA). In some cases, PE- and allophycocyanin-conjugated multimers were added together at this step. The multimer-stained cells were then enriched as described by Moon et al. (18) using anti-PE Ab-coated immunomagnetic beads. The resulting enriched fractions were stained with anti-CD14, CD16, CD19, CD3, CD4, CD8, CD45RO, CD11a, CD27, BV17, or -AV2-labeled mAb and 7-aminoactinomycin D (BD Biosciences). Stained samples were then collected on a Canto II flow cytometer (BD Immunocytometry Systems, San Jose, CA) and analyzed using FlowJo software (Tree Star, Ashland, OR). Counting beads (Life Technologies, Rockville, MD) were used to make sure that the entire cell sample had been collected. The total number of multimer-positive naive CD8 or CD4 T cells was divided by the total number of naive CD8 or CD4 T cells within 10^5 PBMCs to calculate the frequency of Ag-specific naive T cells.

**Cell sorting**

To obtain Ag-specific T cell lines, multimer-positive CD4 or CD8 T cells were sorted by an Aria flow cytometer (BD Immunocytometry systems) following multimer-based enrichment. Cells were then expanded in vitro under nonspecific conditions using rIL-2 (150 U/ml), leuкоagglutinin (1 μg/ml), irradiated PBMCs, and B lymphoblastoid cells as described previously (28). T cell lines were maintained in RPMI 1640 medium supplemented with 10% FBS, 1 mM l-glutamine, and 150 U/ml rIL-2. Purity and specificity of T cell lines were checked by multimer staining and functional assays. Ag-specific CD4 or CD8 T cell clones were obtained from these T cell lines by limiting dilution as described previously.

**Functional assays**

For intracellular cytokine staining, 3 × 10^5 lymphocytes were incubated with TAP-deficient HLA-A*0201–positive T2 cells loaded with 10 μM peptides (E:T ratio: 1:1). Protein-secretion inhibitor brefeldin A (1 mM) was added after 2 h of incubation. Intracellular staining assays were performed as described previously (29).

For degranulation assays, anti-CD107 Abs were added to T cells at the beginning of the incubation time with T2 cells loaded with peptides.

**Statistical analysis**

Data were analyzed, and log_{10} regressions calculated using GraphPad Prism 4.0 software (GraphPad, San Diego, CA). Two-tailed unpaired t tests (95% confidence interval) were performed to compare frequencies of naive Ag-specific T cells. Two-tailed, nonparametric Mann-Whitney tests (95% confidence interval) were performed to compare data distributions: phenotypes of Ag-specific T cells in seronegative donors (n = 10) and percentage of high-avidity naive T cells among Ag-specific naive T cells.

**Results**

**Analysis of CD8 T cells specific for defined pMHC complexes in naive donors**

The features of CD8 T cells specific for viral and tumor Ag restricted by the common HLA-A*0201 allele (referred to as A2) were studied within PBLs from “naive” (i.e., healthy and seronegative) individuals expressing or not this MHCI allele. We selected the following A2-restricted epitopes with previously reported immunodominance: MelA27 (a melanoma-derived Ag) (30), PGT178 (a MelA27–related endogenous epitope derived from the PG transporter) (31), NS3 1073 (29), pp65 295 (32), Gag 77, and MP 58 (33) derived, respectively, from HCV, HCMV, HIV, and influenza virus.

The ex vivo frequency of specific CD8 PBLs was assessed after immunomagnetic enrichment using soluble fluorescent multimers of A2/peptide complexes (“A2 multimers”) with reduced nonspecific binding to CD8 T cells (Fig. 1) (23, 24). Although barely or undetectable within unsorted PBLs from most donors, A2 multimer + T cells were detected in all A2+ individuals after enrichment, irrespective of donor serological status (Fig. 2A and data not shown). Proper Ag specificity of A2 multimer + T cells was supported by their binding to relevant but not irrelevant A2/peptide multimers (Fig. 2B), by their activation by relevant Ag peptides only (Supplemental Fig. 1A), and by their TCR repertoire features. In particular, in line with previous studies reporting biased TCR AV2 and BV17 usage by MelA27+ and MP58-specific T cells respectively (34, 35), ~80% of A2/MelA27+ PBLs were stained by AV2-specific mAb, and ~50% of A2/MP 58+ PBLs were BV17+ (Supplemental Fig. 1B). Unexpectedly, although recognition of PGT178 by some MelA27–specific T cell clones was previously described (31), the corresponding A2/PGT178+ and A2/MelA27 multimers stained distinct PBL subsets with limited or no overlap (Fig. 2B and data not shown). Finally, sensitivity and accuracy of the pMHC-based enrichment process were validated by 1) quantitation of A2-specific T cell clones serially diluted within 10^5 PBLs (Supplemental Fig. 2A), 2) estimation of the residual frequency of A2 multimer + PBLs after immunomagnetic pMHC-based depletion (Supplemental Fig. 2B), and 3) direct comparison of the frequency of A2 multimer + cells estimated in PBLs before and after enrichment in seropositive donors (Supplemental Table I).

**Heterogeneous frequencies of A2-restricted naive CD8 T cells against self- and viral Ags in A2+ donors**

The overall average frequencies of specific subsets within CD8 T cells from A2+ naive donors ranged from 5 × 10^{-6} for Gag 77+ specific to 5 × 10^{-4} for MelA27+ and PGT178-specific T cells.
They also varied for a given Ag specificity from one individual to another, more particularly within the least frequent T cell subsets (Fig. 3A). Although donors included in our study were all preimmune individuals for the tested Ag, specific memory cells were found in every case (Fig. 3B). Because memory cells may have been subject to peripheral expansion, which can introduce a bias into the analysis of Ag-specific T cell frequencies, we specifically tracked naive subsets, as defined by their CD45RO−CD11aloCD27hi phenotype (Fig. 1) (36). Although the frequencies of A2-restricted naive CD8 T cells still showed up to a 100-fold difference from one Ag to another in A2+ donors, they were highly conserved for a given Ag specificity within A2+ donors, irrespective of their other HLA alleles (Fig. 3C). Therefore, the size of A2-restricted T cell subsets seems tightly constrained and barely or not affected by T cell alloreactivity for non-A2 alleles.

A2-restricted CD8 T cells from A2− individuals show generally lower frequencies but similar functional features than their A2+ counterparts

A2 multimer+ CD8 T cells were detected in all A2− donors as well at frequencies that followed the same hierarchy than in A2+ donors (i.e., MelA27 = PGT178 > NS31073 > pp65495 = Gag77) (n = 5; r² = 0.94; p = 0.017) (Fig. 4). Relevance of A2 multimer binding to HLA-A2− T cells was supported by both repertoire and functional analyses. Indeed, HLA-A2− naive CD8 T cells stained by MelA27- and MP58-loaded A2 multimers had similarly biased expression of TCR AV2 and BV17 regions, respectively, than their HLA-A2+ counterparts (Fig. 5A). Moreover, sorted NS31073− and MelA27− specific T cells from A2− donors recognized in vitro A2+ target cells loaded with relevant but not irrelevant peptides in cytotoxicity and cytokine production assays (Fig. 5B and data not shown).

Although the frequencies of pp65495− and Gag77− specific subsets were similar in all donors, irrespective of their A2 haplotype, they were 3–10 times higher in A2+ than in A2− individuals for NS31073−, MelA27−, and PGT178−specific T cells (Fig. 4). Therefore, A2 expression has a heterogeneous impact on the size of A2-restricted T cells subsets, depending on their fine Ag specificity.

Because the alloreactive and self-restricted T cell repertoire might show qualitative differences, notably in terms of avidity (37), this parameter was further studied using engineered A2 multimers unable to engage CD8 coreceptors (referred to as “CD8-null” A2 multimers), which bind to high- but not low-avidity CD8 T cell subsets (25, 26). The percentage of MelA27-, PGT178-, and NS31073−specific T cells stained by CD8-null A2 multimers were similar in all donors, irrespective of their A2 phenotype (Fig. 5C). These results are in line with a previous study showing similar proportions of high-avidity T cells within MelA27−specific T cell clones derived from A2+ versus A2− individuals (38) and extend

FIGURE 1. Multimer-based enrichment strategy. A2 multimer enrichment was performed on 10⁸ freshly isolated PBMCs. Dump−CD3+ gated events were analyzed for CD8 or CD4 expression. A2 multimer+ events were analyzed within either CD4−CD8+ or CD4+CD8− subsets. The frequency of naive A2 multimer+ T cells was further assessed within the CD45RO−CD11aloCD27hi subset. A representative staining obtained following MelA27-multimer enrichment is shown. The dump channel corresponds to pooled events positive for either 7-aminoactinomycin D (dead cells), CD19 (B cells), CD16 (NK cells), or CD14 (monocytes).

FIGURE 2. Detection of A2 multimer+ T cells after multimer-based enrichment. A, Representative contour plots of A2 multimer versus CD3 on Dump−CD3−CD4−CD8− gated events. Results are shown either before (top row, analysis of 5 × 10⁵ cells) or after (bottom row, analysis of the whole sample) multimer-based enrichment of 10⁸ PBMCs from A2+ donors seronegative for HCV, HIV, and HCMV. Frequencies of multimer+ cells are indicated. B, Representative stainings with A2 multimers multimerized with PE- and APC-labeled streptavidin and loaded with either distinct or identical antigenic peptides within Dump−CD3−CD4−CD8− gated cells after simultaneous enrichment with the corresponding multimers in A2+ donors.
Impact of A2 expression on naive Ag-specific CD8 T cell expression

The frequency of A2 multimer+ naive CD4 T cells ranged from $5 \times 10^{-8}$ to $5 \times 10^{-7}$, depending on the Ag/peptide complex studied. Although such frequencies were much lower within CD4 than CD8 T cells for most A2-restricted Ag studied, they were in the same range for pp65-specific CD4 T cells, irrespective of their coreceptor phenotype and A2 haplotype (Fig. 7A, 7B). Unexpectedly, the frequency of A2 multimer+ CD4 T cells correlated with the frequency of CD8 T cells specific for the corresponding pMHC complexes in A2+ and A2− donors (Fig. 7C). Although CD4/CD8 double-positive T cells were strictly gated out, the above correlation could reflect coreceptor modulation on a subset of naive CD8 T cells occurring through yet undefined mechanisms after their intrathymic selection. To address this, we assessed the impact of MHC class I expression on the frequency of A2-restricted CD4 T cells. Unlike their CD8 counterparts, the frequency of A2-restricted CD4 T cells is not affected by MHC class I expression

Figure 4. Impact of A2 expression on naive Ag-specific CD8 T cell frequencies. Comparison of naive Ag-specific CD8 T cell frequencies in HCV-, HCMV-, and HIV-seronegative donors expressing (black symbols) or not (open symbols) A2. Horizontal bars indicate mean value. The p values were calculated using two-tailed unpaired t tests (n = 10). ns, not significant.

MHC class I-restricted CD4 T cells were previously reported (39, 40), but their frequency, origin, molecular, and functional relationships with their CD8 counterparts could not be studied in detail thus far. Like their CD8 counterparts, CD4 T cells stained by MelA2/−/−A2 multimers predominantly expressed Av2.2 TCRs (Fig. 6B). The functional relevance of these A2 multimer+ CD4 subsets was supported by their specific binding to A2 multimers loaded with relevant peptides only (Fig. 6C). Moreover, A2 multimer+ CD4 T cell lines and clones were stimulated by their cognate but not irrelevant peptide Ag (Fig. 6D). Interestingly, they produced much more IL-2 than their CD8 counterparts after Ag stimulation, consistent with a T helper-like phenotype (Supplemental Fig. 3 and data not shown).

Unlike their CD8 counterparts, the frequency of A2-restricted CD4 T cells is not affected by MHC class I expression

The frequency of A2 multimer+ naive CD4 T cells was stained by A2 multimers in all donors (Fig. 6A). Such conclusion was based on our observation that the low but detectable frequency of CD4+CD8− T cells within naive and memory compartments was similar in A2+ and A2− donors. This is in line with previous studies showing that the frequency of A2-restricted CD4 T cells is not affected by MHC class I expression (41). In line with previous studies, TAP-deficient donors (Fig. 8A). This indicated that A2 multimer+ CD4 T cells were not selected by MHC class I molecules.

Characterization of CD4 peripheral T cells specific for A2/peptide complexes

Besides CD8 T cells, a low but detectable fraction of CD4+CD8− T cells was stained by A2 multimers in all donors (Fig. 6D). Such frequency was 5- to 10-fold more frequent within naive T cells from A2+ than A2− individuals (Fig. 5D). Therefore, although the scarcity of pp65-specific and Gag77-specific T cells precluded detailed assessment of their avidity, these results indicated that the naive T cell repertoire of A2+ donors is skewed toward high-avidity recognition of several self-, tumor, and viral Ags restricted by A2.
Similar frequencies of A2-restricted CD4 and CD8 T cells in A2− individuals

The higher frequency of A2-restricted CD8 than CD4 T cells in A2− individuals could be explained by either an MHC class I recognition bias of the CD8 T cell repertoire because of selection by non-A2 MHC class I alleles, or alternatively, it could merely reflect enhanced avidity of CD8 T cells for MHC class I complexes, conferred by expression of the proper coreceptor. To address this, we compared the frequencies of specific CD4 and CD8 T cells using A2 multimers unable to engage CD8 coreceptors. Although much higher in CD8 than CD4 T cells in A2+ donors (Fig. 8B, upper panel), the frequencies of MelA27-, PGT179-, and NS31073-specific T cells stained by CD8-null multimers were barely higher in CD8 than in CD4 subsets from A2− individuals (Fig. 8B, lower panel). Thus, the presence of A2-restricted CD8 T cells in A2− donors seems primarily explained by cross-reactivity for the corresponding Ags of T cells selected by unrelated or weakly related pMHC complexes.

Discussion

Except for MelA27-specific T cells, whose frequency, phenotype, and repertoire features in naive individuals were previously described (38, 43), little was known thus far about the size and features of naive Ag-specific T cells in humans. In this regard,
the current study provides a first estimate of the frequency range of naive CD8 T cells against several immunodominant Ag in adults. The frequencies of subsets reacting against distinct A2-restricted Ag of TCRs selected by MHC class I molecules. Also in agreement with murine studies, frequencies of human CD8 T cells specific for a given Ag showed limited interindividual variations. Such a restrained behavior was less expected in humans, owing to their heterogeneous immunological history and MHC background.

The proportions of naive CD8 T cells within cell subsets specific for a given pMHC complex greatly varied from one Ag specificity to another in seronegative A2+ individuals. Moreover, such proportions correlated with the frequency of naive CD8 T cells directed against the corresponding Ag (n = 50; $\rho^2 = 0.535; p < 0.001$). The presence of Ag-specific memory cells in unprimed individuals could reflect either stimulation by cross-reactive Ag or, as suggested by recent studies in mice, a phenotypic switch of Ag-unexperienced cells after homeostatic expansion (44). This might lead to heterogeneous exhaustion or reduction of the naive compartment, depending on the Ag specificity studied, and therefore could explain the heterogeneous frequencies of naive-specific T cell subsets observed in our study. However, T cells directed for instance against Gag77 versus MelA27 showed on average a 100-fold frequency difference but only a 2-fold difference in terms of percentage of naive T cells. Therefore, other mechanisms likely account for such frequency differences. Homeostatic expansion of naive cells is probably not involved in this study because one of the most frequent naive CD8 subset, specific for MelA27, was previously shown to carry high numbers of TCR recombination excision DNA circles (45), indicating limited proliferation after thymic selection and exit.

Analysis of CD4 T cells specific for various A2/peptide complexes provided possible insights into the mechanisms contributing to the heterogeneity of Ag-specific naive CD8 T cell frequencies. We obtained strong evidence that A2-restricted CD4 cells were not selected by HLA class I molecules and thus that A2 multimer binding to CD4 T cells reflected cross-reactivity for A2-restricted Ag of TCRs selected by MHC class II molecules. The frequencies of A2 multimer+ CD4 T cells were in the same range than those of CD8 T cells stained by A2 multimers unable to engage CD8 coreceptors in A2− donors. This supports the assumption that A2 multimer binding in both cases reflects inherent cross-reactivity for A2/peptide complexes of the naive T cell repertoire, irrespective of its selection context. The peripheral repertoire is thought to be made of $25–100 \times 10^6$ distinct clonotypes totaling $\sim 10^{12}$ T cells (1). With an estimated frequency of A2-restricted CD4 T cells of $5 \times 10^{-6}$ to $5 \times 10^{-5}$, 20–200 distinct TCRs should cross-react to a given A2/peptide complex in a coreceptor-independent fashion and $\sim 10$-fold more with the help of the CD8 coreceptor. Such estimates are clearly in the range of previously predicted values (46) and are consistent with the view that TCRs show a high level of cross-reactivity. Importantly, the correlation between the frequencies of CD4 and CD8 T cells specific for a given A2/peptide complex would indicate that the size of naive A2-restricted CD8 T cells is determined at least in part by the cross-reactivity level of the TCR repertoire.
riphery. Accordingly, the low frequency of pp65495-specific interactions with selecting endogenous pMHC complexes and repertoire remodeling, depending on the avidity of thymocyte of positive versus negative intrathymic selection in the context of a natural infection. In this particular case, intrathymic selection of T cell precursors by a given MHC allele seems dispensable for the generation of a functional high-avidity and peptide-specific peripheral response restricted by the same or a related MHC allele or even from a distinct MHC class. Therefore, in light of the distinct functional -specialization of MHC class I- and class II-restricted T cells, the driving force of intrathymic positive selection might not be linked to intrathymic selection of T cell precursors by a given class of MHC complexes and their structural relationships with pMHC complexes and seronegative individuals. By contrast, nondegenerate high-affinity interactions with low-density A2/peptide complexes closely related or identical to MelA27/A2 or PGT178/A2 could explain the much higher frequency of MelA27- or PGT178-specific T cells in A2+ than in A2- individuals and their low cross-reactivity, reflected by their predominantly naive phenotype in seronegative healthy donors. Irrespective of this issue, these results, which suggest that the dominant contribution of either positive or negative selection on the shaping of the A2-restricted repertoire will depend on the pMHC complex studied, could reconcile seemingly opposite conclusions drawn from analysis of T cell repertoires selected by a single pMHC complex (8, 11). Further analyses of Ag-specific subsets at different intrathy- mic developmental stages will probably allow more direct validation of these hypotheses in humans.

Although initial frequencies of naive T cell precursors seem to regulate the strength and immunodominance of memory responses in the mouse (17, 19, 20), our study indicates that immunodominance of A2-restricted T cell response in humans is not always associated with a high frequency of naive T cell precursors. In particular, pp65495-specific T cells can make up a large fraction of virus-specific CD8 T cells in A2+ individuals along acute HCMV infection (32, 33) but were found at similarly low frequencies within both naive CD8 and CD4 T cells, irrespective of their A2 phenotype. Moreover, both pp65495-specific CD8 and CD4 T cells were expanded and switched to a memory phenotype in some HCMV-seropositive donors (F. Legoux, X. Saulquin, and M. Bonneville, unpublished observations), suggesting peripheral activation of both subsets in the context of a natural infection. In this particular case, intrathymic selection of T cell precursors by a given MHC allele seems dispensable for the generation of a functional high-avidity and peptide-specific peripheral response restricted by the same or a related MHC allele or even from a distinct MHC class. Therefore, in light of the distinct functional -specialization of MHC class I-and class II-restricted T cells, the driving force of intrathymic positive selection might not be to focus T cells toward recognition of self-MHC but rather to link selection of developing T cells by a given class of MHC molecules to a particular functional program. In this regard, owing to the distinct functional profiles of A2-restricted CD8 and CD4 T cells suggested by our results, the possibility that MHC I-restricted CD4 T cells provide help for CD8 T cell responses specific for the same Ag would certainly deserve further investigations.

Besides inherent TCR cross-reactivity, comparative analysis of A2+ versus A2- donors unveiled a predominantly positive, although heterogeneous impact of the HLA-A2 background on the frequencies of A2-restricted naive T cells, depending on the Ag specificity studied. In line with the correlation between the percentage and frequencies of naive A2-restricted T cells in A2+ -seronegative donors, the percentage of naive T cells also correlated with the ratio of naive T cell frequencies between A2+ and A2- donors, irrespective of their specificity for either natural or artificial A2-restricted peptides (Fig. 9). In other words, the higher the proportion of naive T cells within cell subsets specific for a defined A2/peptide complex in A2+-seronegative individuals, the higher the positive impact of A2 expression on naive T cell frequencies. On the basis of previously proposed models, the above correlation could reflect variable contribution of positive versus negative intrathymic selection to peripheral repertoire remodeling, depending on the avidity of thymocyte interactions with selecting endogenous pMHC complexes and their structural relationships with pMHC encountered in the periphery. Accordingly, the low frequency of pp65495-specific T cells could be explained by their intrathymic selection by poorly related pMHC complexes, hence the lower impact of the A2 background on their frequency and a higher probability to be cross-activated by weakly related pMHC complexes in seronegative individuals. By contrast, nondegenerate high-affinity interactions with low-density A2/peptide complexes closely related or identical to MelA27/A2 or PGT178/A2 could explain the much higher frequency of MelA27- or PGT178-specific T cells in A2+ than in A2- individuals and their low cross-reactivity, reflected by their predominantly naive phenotype in seronegative healthy donors. Irrespective of this issue, these results, which suggest that the dominant contribution of either positive or negative selection on the shaping of the A2-restricted repertoire will depend on the pMHC complex studied, could reconcile seemingly opposite conclusions drawn from analysis of T cell repertoires selected by a single pMHC complex (8, 11). Further analyses of Ag-specific subsets at different intrathymic developmental stages will probably allow more direct validation of these hypotheses in humans.

Although initial frequencies of naive T cell precursors seem to regulate the strength and immunodominance of memory responses in the mouse (17, 19, 20), our study indicates that immunodominance of A2-restricted T cell response in humans is not always associated with a high frequency of naive T cell precursors. In particular, pp65495-specific T cells can make up a large fraction of virus-specific CD8 T cells in A2+ individuals along acute HCMV infection (32, 33) but were found at similarly low frequencies within both naive CD8 and CD4 T cells, irrespective of their A2 phenotype. Moreover, both pp65495-specific CD8 and CD4 T cells were expanded and switched to a memory phenotype in some HCMV-seropositive donors (F. Legoux, X. Saulquin, and M. Bonneville, unpublished observations), suggesting peripheral activation of both subsets in the context of a natural infection. In this particular case, intrathymic selection of T cell precursors by a given MHC allele seems dispensable for the generation of a functional high-avidity and peptide-specific peripheral response restricted by the same or a related MHC allele or even from a distinct MHC class. Therefore, in light of the distinct functional -specialization of MHC class I- and class II-restricted T cells, the driving force of intrathymic positive selection might not be to focus T cells toward recognition of self-MHC but rather to link selection of developing T cells by a given class of MHC molecules to a particular functional program. In this regard, owing to the distinct functional profiles of A2-restricted CD8 and CD4 T cells suggested by our results, the possibility that MHC I-restricted CD4 T cells provide help for CD8 T cell responses specific for the same Ag would certainly deserve further investigations.

FIGURE 8. Analysis of selection modalities of A2 multimer+ CD4 or CD8 T cells from A2- donors. A, Contour plots of A2 multimer+ CD4 T cells in TAP-sufficient versus -deficient donors after multimer-based enrichment from 10^6 PBMCs. Percentages of CD4+ versus CD4- Dump CD3+multimers+ T cells are indicated. Data are representative of two different TAP-deficient donors. B. Comparative analysis of the frequencies of A2 CD8-null multimer-specific T cells within naive CD4 and CD8 compartments. Horizontal bars indicate mean value. The p values were calculated using two-tailed unpaired t tests.

FIGURE 9. Correlation between the impact of A2 expression on the naive T cell frequency and the mean percentage of naive Ag-specific CD8 T cells in A2+ donors. The impact of A2 expression was calculated by dividing the mean frequency of naive CD8 T cells in A2+ donors (n = 10 for each specificity) by the mean frequency of naive CD8 T cells in A2- donors (n = 10 for each specificity). Black symbols indicate data obtained with artificial peptides multimers (p800 and p21). Although p800 and p21 multimers are homologous respectively to MelA27 and NS31073 ones, T cell-specific repertoires showed no overlap (Fig. 4 and data not shown). The p values and r^2 values were calculated using a Pearson’s test (n = 7).
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Disclosures

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References