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Tolerance to Maternal Immunoglobulins: Resilience of the Specific T Cell Repertoire in Spite of Long-Lasting Perturbations

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T cell tolerance is established and maintained through various mechanisms, the critical component being the persistence of the specific Ag. However, at the molecular level, the nature of the recovering TCR repertoire following breakdown of tolerance is unknown. We address this important question by following α light chain constant region (Ck)-specific CD4+ T cells of α light chain knockout (α−/−) mice born to κ+ mothers. These cells, which were in contact with maternal κ+ Igs from early ontogeny until weaning, were strongly tolerated. Tolerance was reversible and waned with the disappearance of peptide Ck134–148 presentation in lymphoid organs, including the thymus. Whereas three specific Vβ1β rearrangements emerged in the peptide Ck134–148-specific CD4+ T cell response of all regular κ+ mice, soon after breakdown of tolerance only one of these rearrangements was detected. The two others displayed a significant delay in reappearance and were still rare at 26 wk of age, while the control proliferative response had already recovered 3 mo earlier. At 52 wk of age, a complete recovery of the three canonical Vβ1β rearrangements was observed. Thus, although profoundly perturbed for several months, the T cell repertoire returns to equilibrium, highlighting the resilient nature of this system. The Journal of Immunology, 1999, 163: 6511–6519.

In response to defined peptide/MHC complexes, highly restricted to extremely diverse TCR repertoires have been shown to be selected (8–12). By analogy with Ids expressed in various Ab responses (see Ref. 13 for a review), some of these T cell responses can be divided into “private” and “public or recurrent” mechanisms (11, 12). The private response involves T cell clones using TCR chain rearrangements which are distinct in different individuals or are expressed in only a few of them. In contrast, the public component of a T cell response involves a defined TCR chain rearrangement which reproducibly emerges in most individuals of a given inbred strain. Peripheral self-tolerance, as well as tolerance to foreign Ags, administered by oral or intravenous routes involves two main mechanisms: “recessive” mechanisms, which are characterized by deletion or anergy, and “dominant” mechanisms, which involve the selection of regulatory T cells (5, 14–18).

In euthymic mice, tolerance to soluble Ag is never permanently acquired (19–22) even if large amounts of Ag are delivered early in ontogeny (20, 22). Both a decrease in Ag concentration (21, 22) and new thymic emigrants (23) were shown to be responsible for the breakdown of tolerance. Transfer experiments in athymic or euthymic irradiated mice revealed that T cell tolerance induced against membrane-associated Ag is also reversible in the absence of nominal Ag (24–26).

In the B cell compartment, early idiotypic manipulations via maternal immunization with Ag or Ids or after treatment of newborns with anti-idiotypic Abs were shown to induce a profound state of suppression of the particular Id or even of the Ab response for various periods of time. Apart from two idiotypic systems in which no kinetic studies are available (27, 28), it has been shown that although suppression of Ab responses is always reversible, its recovery is associated with the expression of the same (29) or different idiotypic repertoires (30–34).

In contrast to what is known about the idiotypic repertoire of B cells recovering from tolerance, the nature of the reemerging T cell repertoire has not been studied.
Therefore, the questions we addressed in this paper were: does a completely different repertoire appear along with recovery of the T cell response or is the T cell repertoire a resilient system, i.e., does it return to a position of equilibrium as described for most ecological systems when subjected to disturbance (35)?

To answer these questions we followed the $\kappa$ light chain constant region (C$\kappa$)-specific T cell response and repertoire of $\kappa$ knock-out ($\kappa^-/\kappa^-$) mice born to $\kappa^+/\kappa^+$ mothers after breakdown of tolerance. We have previously shown that, in $H^2d$ $\kappa^+/\kappa^+$ mice born to $\kappa^+/\kappa^-$ mothers (regular $\kappa^+/\kappa^-$ mice), $\kappa$ light chains induce a diverse C$\kappa$-specific CD4$^+$ T cell response which recognizes one single peptide C$k_{134-148}$ (Ck peptide) (Ref. 36, and unpublished data).

In the present report, we show that maternal $\kappa$ positive Igks (Igks) strongly but transiently influence the offspring’s C$\kappa$-specific CD4$^+$ T cell proliferative response. Indeed, $\kappa^+/\kappa^-$ mice born to $\kappa^+/\kappa^-$ mothers display a state of C$\kappa$-specific CD4$^+$ T cell tolerance which wanes with the disappearance of Ck peptide at the surface of their spleen, lymph node, and thymus cells. The comparison of the repertoires of C$\kappa$-specific CD4$^+$ T cells of regular $\kappa^+/\kappa^-$ mice and those of $\kappa^+/\kappa^-$ mice born to $\kappa^+/\kappa^-$ mothers was conducted making use of the Immunoscope method described by Pannetier et al. (37) and sequence analysis. Our data show that the CD4$^+$ C$\kappa$-specific T cell response in $H^2d$ regular $\kappa^+/\kappa^-$ mice is characterized by the expression of three distinct public $V\beta$ rearrangements. A kinetic analysis of the $V\beta$ repertoire of $\kappa^+/\kappa^-$ mice born to $\kappa^+/\kappa^-$ mothers clearly indicates that maternal Igks induce long-lasting but reversible modifications in the T cell repertoire, highlighting the resilient nature of this repertoire.

Materials and Methods

Mice

H$^2d$ $\kappa^+/\kappa^-$ mice were used in this study and in our previous study (36). Briefly, $H^2d$ $\kappa^+/\kappa^-$ mice were obtained using $\kappa$-deficient 129 mice ($\kappa^+/\kappa^-$, $H^2d$) generated by targeted mutation in the $\kappa$ gene from 129/Sv embryonic stem cells (38) as follows: mice with the $\kappa^+/\kappa^-$, $H^2d$-phenotype were selected in the $F_2$ generation obtained by crossing between (BALB/c x 129 $\kappa^+/\kappa^-$)F1 mice. These individuals were bred by brother/sister crossings, and mice belonging to the seventh generation were used. $\kappa^+/\kappa^-$ mice born to $\kappa^+/\kappa^+$ mothers were identified by FACS analysis at 3–4 wk of age, as described previously (36).

Proliferation assays

Mice (4–26 wk old, as indicated in figure and table legends) were immunized in the hind footpads with 10$^5$ cells/well of 96-well culture plates. T cell hybridomas and tissue localization of C$\kappa$ peptide

T cell hybridomas and tissue localization of C$\kappa$ peptide

T cell hybridomas M44-C2 and M67-C11 were previously described (36). The stimulation assays were performed with 10$^3$ hybridoma cells which were cultured with irradiated nonpurified cells (extracted from the indicated lymphoid tissues) from $\kappa^+/\kappa^-$ mice born to $\kappa^+/\kappa^-$ mothers, in 96-well tissue culture plates. After 24 h, secretion of IL-2 in supernatants was measured by proliferation of IL-2-dependent CTLL-2 cells after $[^3H]$Tdr incorporation as previously described (36).

RNA extraction and cDNA synthesis

The C$\kappa$-specific public T cell repertoire was determined from individual H$^2d$ $\kappa^+/\kappa^-$ mice born to $\kappa^+/\kappa^+$ mothers immunized with Ck peptide in CFA. mRNA from lymph node cells (LNC) stimulated for 4 days in 2 ml at 2.5 x 10$^5$ cells/ml with 25 $\mu$g/ml of Ck peptide or PPD (as described above) was extracted using Trizol (Life Technologies, Grand Island NY), according to the manufacturer’s instructions. After two steps of CD8$^+$ T cells using the biotinylated anti-CD8 1R.4 5 Ab (39) and streptavidin M-280 Dynabeads (N-0212; Dynal, Oslo, Norway). RNAs were reverse transcribed into cDNA using a cDNA synthesis kit (Boehringer Mannheim, Mannheim, Germany).

Oligonucleotides

Oligonucleotides used for Immunoscope studies and sequence analyses were previously described (37).

Determination of the C$\kappa$-specific public T cell repertoire with Immunoscope

The Immunoscope technique initially developed by Pannetier et al. (37) allows the detection of clonal T cell expansions in a complex mixture of the repertoire, which consists of two after depletion of CD8$^+$ T cells using the biotinylated anti-CD8 1R.4 5 Ab (39) and streptavidin M-280 Dynabeads (N-0212; Dynal, Oslo, Norway). RNAs were reverse transcripted into cDNA using a cDNA synthesis kit (Boehringer Mannheim, Mannheim, Germany).

An aliquot of each of the specific V$\beta$-CB4 PCR products (V$\beta$2-, V$\beta$6-, and V$\beta$13-CB4) was amplified by 25 cycles with their specific V$\beta$ and J$\beta$ primers and was submitted to run-off reactions with different labeled J$\beta$ oligonucleotides and are size fractionated on polyacrylamide gels. Six to eight peaks corresponding to various CDR3 sizes, each spaced by 3 nt, reflect the length of in-frame transcripts. A typical bell-shaped distribution of CDR3 lengths is observed in naive mice. After immunization, specific clonal proliferation leads to significant modification of some profiles with expansion of one or a few peaks.

PCR were conducted in 40 $\mu$L on 1/40 of the cDNA using 2 U of Taq polymerase (Eurogentec, Seraing, Belgium) in the supplier’s buffer with a V$\beta$ specific sense primer and the CB4 antisense primer as previously described (12). To cover the full repertoire, PCR were conducted using the primers specific for the 23 functional $V\beta$ segments of BALB/c. Forty cycles were performed involving first a 30-s denaturation step at 94°C, a 45-s annealing step at 60°C, and a 45-s elongation step at 72°C. Each amplified product was then used as a template for the elongation reaction with a C$\kappa$ oligonucleotide (CB5′) labeled with a fluorescent tag (run-off reactions) (12). The fluorescent run-off products were loaded on polyacrylamide gels and subjected to electrophoresis in an automated DNA sequencer. The CDR3 size distribution and signal intensities were then analyzed with Immunoscope software designed for this purpose (37). The PCR products for which a significant peak increase was recurrently observed in various regular H$^2d$ $\kappa^+/\kappa^-$ mice after restimulation with Ck peptide only, but not after PPD restimulation, were subjected to run-off reactions using all 12 J$\beta$-labeled specific primers.

Sequences

An aliquot of each of the specific V$\beta$-CB4 PCR products (V$\beta$2-, V$\beta$6-, and V$\beta$13-CB4) was amplified by 25 cycles with their specific V$\beta$ and J$\beta$ primers and was submitted to an elongation step at 72°C for 10 min. Three different sequencing strategies were conducted. 1) Direct sequencing of PCR products: 5 $\mu$L of the V$\beta$-J$\beta$ amplifications were treated with 0.25 $\mu$L of exonuclease at 10 U/$\mu$L (Amersham, Orsay, France) and with 0.25 $\mu$L shrimp alkaline phosphatase (Amersham) at 1 U/$\mu$L for 40 min at 37°C and then for 20 min at 80°C. Sequences were then conducted with the corresponding V$\beta$ primers using the Big Dye Terminator kit (Perkin-Elmer, Foster City, CA) according to the manufacturer’s instructions. Sequences were run on a model 377 DNA sequencer (Perkin-Elmer) and were analyzed according to expected CDR3 size. 2) Sequencing after cloning of PCR products: 5 $\mu$L of the V$\beta$-J$\beta$ amplifications were cloned using the TOPO TA cloning kit (Invitrogen, Carlsbad, CA) according to the manufacturer’s recommendations. Individual white colonies were picked, boiled at 90°C for 15 min in 20 $\mu$L distilled water, and the inserts were directly PCR-amplified in 40 $\mu$L using M13 reverse and M13-40 reverse primers. After exonuclease and shrimp alkaline phosphatase treatment, sequencing reactions were conducted in the presence of M13-20 primer using the Big Dye Terminator kit. 3) Sequencing after cloning the band of interest cut out from a polyacrylamide gel: this strategy was only used for V$\beta$2-J$\beta$2 rearrangements with the specific primers. PCR products were ethanol precipitated and loaded on an 8% polyacrylamide, 7 M urea gel. After migration, PCR products were visualized by silver staining of the gel (DNA silver staining system, Promega, Madison, WI). The band corresponding to
Ck-specific T cell proliferative responses. A. Four-wk-old H-2b/k−/− regular mice (■), k−/− mice born to k+/− mothers (□), or k+/− mice (□) were immunized with 10 μg/mouse of Ck peptide. Eight days later, popliteal LNC were cultured with 25 μg/ml of each indicated Ag. Cultures were assayed for proliferation after 4 days by [3H]TdR uptake added for the last 13 h of culture. Data are expressed as means of triplicate cultures minus background values (medium alone) ± SD. Each histogram corresponds to the response of three mice. B. The CD4+ T cell response from k−/− mice born to k+/− mothers (□, thick curve) and k+/− mice (○, thin curve) immunized with Ck peptide at 4, 6, 8, 12, and 20 wk of age, was determined as in A. Each circle represents an individual mouse, and the polynomial curve is drawn through the mean value for each age. Data are expressed according to the percentage of k light chain-specific response in comparison to the PPD-specific response (k light chain and PPD were used at 25 μg/ml) for each individual mouse as described in Materials and Methods. The response of 4- or 26-wk-old regular k−/− mice was 61 ± 4%.

FIGURE 1. Ck-specific T cell proliferative responses. A. Four-wk-old H-2b H-2b k−/− regular mice (■), k−/− mice born to k+/− mothers (□), or k+/− mice (□) were immunized with 10 μg/mouse of Ck peptide. Eight days later, popliteal LNC were cultured with 25 μg/ml of each indicated Ag. Cultures were assayed for proliferation after 4 days by [3H]TdR uptake added for the last 13 h of culture. Data are expressed as means of triplicate cultures minus background values (medium alone) ± SD. Each histogram corresponds to the response of three mice. B. The CD4+ T cell response from k−/− mice born to k+/− mothers (□, thick curve) and k+/− mice (○, thin curve) immunized with Ck peptide at 4, 6, 8, 12, and 20 wk of age, was determined as in A. Each circle represents an individual mouse, and the polynomial curve is drawn through the mean value for each age. Data are expressed according to the percentage of k light chain-specific response in comparison to the PPD-specific response (k light chain and PPD were used at 25 μg/ml) for each individual mouse as described in Materials and Methods. The response of 4- or 26-wk-old regular k−/− mice was 61 ± 4%.

FIGURE 2. Kinetics of presentation of maternal IgG-derived Ck peptide in various lymphoid organs. A total of 106 mesenteric lymph nodes (A), 0.5 × 106 splenic (B), or 2 × 106 thymic (C) irradiated cells (10 Gy) from k−/− mice born to k+/− mothers of various ages were cultured with 105 cells of the Ck-specific T cell hybridoma M67-C11 (36). After 24 h of culture, IL-2 production was determined by adding 100 μl aliquots of culture supernatants to 104 CTLT for 3 days. Results are expressed as average cpm of triplicate culture ± SD of [3H]TdR incorporation added for the last 6 h of CTLT culture. Similar results were obtained with another Ck-specific T cell hybridoma, M44-C2 (Ref. 36, and data not shown). Supernatants from M67-C11 and M44-C2 T cell hybridomas cultured in the presence of lymphoid cells from k−/− pups born to regular k−/− mice induced less than 500 cpm of CTLT proliferation.

Results

Ck-specific CD4+ T cell tolerance in k−/− mice born to k+/− mothers

To determine the influence of maternal Ig on the T cell progeny, we assessed the Ck-specific T cell response of k−/− mice born to k+/− mothers. In this situation, mice were exposed to large amounts of Igκ from fetal life to weaning. The proliferative activity of both k light chain- and Ck peptide-restimulated LNC of k−/− mice born to k+/− mothers immunized at 4 wk of age with Ck peptide is totally abolished when compared with that of age-matched immunized regular k−/− mice and is similar to that of k+/− mice (Fig. 1A). This tolerance is specific inasmuch as the response to PPD is not altered (Fig. 1A). Our previous observations have indicated that maternal Igκ are able to induce tolerance in CD8+ T cells from offspring that are unable to synthesize k light chains. The present work extends the analysis to the Ck-specific CD4+ T cell compartment (22).

The next experiment was designed to assess the kinetics of tolerance breakdown. To this end, k−/− mice born to k+/− mothers were immunized at different ages with Ck peptide. Although no proliferation of Ck-specific CD4+ T cells was elicited at 4 wk of age, CD4+ T cell reactivity reappears 6 wk after birth and returns to normal by 12 wk after birth (Fig. 1B). We then followed the tissue localization of Ck peptide-presenting cells and the kinetics of presentation of the Ck peptide naturally processed by cells of tolerantized mice using Ck-specific T cell hybridomas. Fig. 2 shows that maternal Igκ-derived Ck peptide is not only presented on the surface of mesenteric lymph node cells (Fig. 2A), one of the first lymphoid tissues in which T cells encounter peptides from orally administered proteins, but is also presented very efficiently by

the 7-residue-long CDR3β was cut out from the gel and submitted to a second PCR of 20 cycles using the same primers. The second PCR product was cloned and sequenced as described above.
both splenic and thymic cells (Fig. 2, B and C, respectively). For each of the three lymphoid tissues, similar kinetics of presentation are observed, with maximal presentation around 3 wk of age after which a sharp drop in activity occurs as soon as 4 wk.

Taken together, these data show that tolerance of Cκ-specific CD4⁺ T cells under physiological conditions is very strong but reversible, and the data also extend our previous observations (22) in showing that there is a temporal correlation between the reversion of the CD4⁺ T cell tolerance and the disappearance of Cκ peptide presentation.

**Characterization of the Cκ-specific CD4⁺ T cell Vβ public repertoire in regular κ⁻/⁻ mice**

As the dissection of the recovering T cell repertoire of κ⁻/⁻ mice born to κ⁺/⁻ mothers after breakdown of tolerance was possible only for the public component of the Cκ-specific T cell response, we first sought to identify such a response in mice that have never been in contact with κ light chains. To this end, LNC from regular κ⁻/⁻ mice immunized with Cκ peptide in CFA were restimulated in vitro with Cκ peptide, κ light chain, or PPD. After a 4-day incubation period, proliferating cells were depleted of CD8⁺ T cells and the CDR3β size distribution of the remaining T cells was analyzed with the Immunoscope technique (37).

Vβ-Cβ elongation products in which a peak of a given CDR3 size was significantly and specifically increased in all primed κ⁻/⁻ animals were subjected to run-off reactions with each of the 12 Jβ-labeled specific oligonucleotides. For all immunized regular κ⁻/⁻ mice, we constantly observed the emergence of three recurrent Vβ-Jβ rearrangements. In Fig. 3, a typical experiment obtained from 1 of 12 κ⁻/⁻ mice is represented. A significant increase of a peak corresponding to a CDR3 of 7 aa in size for the Vβ2 (Fig. 3, panel 1) and of 9 aa for the Vβ6 (panel 3) and Vβ13 (panel 5) was found in run-off reactions conducted with the Cβ5’-labeled primer. The increase in the height of these peaks was due to the recurrent rearrangements of Vβ2 with the Jβ2.7 segment (panel 2), of Vβ6 with Jβ1.4 (panel 4), and of Vβ13 with Jβ2.3 (panel 6). These Vβ rearrangement peaks were strictly specific for the Cκ peptide, as is shown by the fact that none of them were observed at the expected CDR3 size in identical run-off experiments conducted with PPD-primed regular LNC from Cκ peptide-primed κ⁻/⁻ mice (panels 13–18). Moreover, the recurrent Cκ-specific T cell rearrangements were elicited against the naturally processed Cκ peptide because identical Immunoscope profiles were observed whether LNC were restimulated in vitro with Cκ peptide or with native κ light chain (panels 7–12). Although Cκ peptide used for immunization and in vitro restimulation is synthetic, these data suggest that it contains only epitopes expressed by the naturally processed Cκ peptide region.

To confirm the public nature of the recurrent rearrangements, we directly sequenced the PCR products of in vitro restimulated LNC from mice of different ages. An identical CDR3 of 7 aa with the SADNYEQ aa sequence was found in the Vβ2-Jβ2.7 rearrangement from all mice (Table I). A recurrent CDR3 with the expected size of 9 residues and the SIGGSNERL aa sequence was detected in the Vβ6-Jβ1.4 rearrangement (Table I). It should be noted that a second sequence (SRTGSNERL) was found in some bacterial colonies containing the Vβ6-Jβ1.4 insert from some κ⁻/⁻ mice (data not shown). Finally, two “synonymous” CDR3 sequences were found from the recurrent Vβ13-Jβ2.3 rearrangement: of 12 individual κ⁻/⁻ mice, 6 displayed the SFAGRAETL amino acid sequence and 6 displayed the SWGGRAETL CDR3 amino acid sequence (Table I). All these public CDR3 sequences were also found in LNC of Cκ peptide-primed κ⁻/⁻ mice restimulated with κ light chain (Table I, mice L1 and L3). Taken together, these data indicate that part of the CD4⁺ T cells specific for a peptide naturally derived from Cκ uses three distinct public Vβ rearrangements during at least half of the mouse’s life.

![FIGURE 3. Profiles of the fluorescent Vβ-Cβ and Vβ-Jβ run-off products obtained from Cκ peptide-primed regular κ⁻/⁻ mice. Eight-week-old mice were immunized with Cκ peptide in CFA. LNC were harvested 8 days later and restimulated in vitro with Cκ peptide (left), κ light chain (center), or PPD as control (right). After 4 days in culture, total RNA from proliferating lymphocytes depleted of CD8⁺ T cells were extracted and reverse transcribed as described in Materials and Methods. The cDNAs were amplified with the various sense Vβ and antisense Cβ primers. Fluorescent antisense Cβ (Cβ5’) or Jβ primers were used for run-off reactions to reveal specific clonal expansion. The run-off products were size fractionated in an automated DNA sequencer. Of the 23 Vβ-Cβ combinations analyzed, only three showed recurrent peak expansions and they are represented in this figure. Thick arrows indicate Cκ peptide-specific peaks. Thin arrows show control peaks. The horizontal axis shows the size in amino acids of the CDR3 junctional regions deduced from the fragment size. The vertical axis shows the fluorescent intensity in arbitrary units.](http://www.jimmunol.org/Downloaded_from/pic303x540.png)
Table I. Public CDR3β amino acid sequences from Cκ-immunized 8- to 52-wk-old regular κ−/− mice in response to Cκ peptide or κ light chain

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Influence of maternal Igκ on the repertoire of Cκ-specific CD4+ T cells

To determine the influence of maternal Igκ on the developing T cell repertoire of the offspring, we analyzed the functional Cκ-specific CD4+ T cell repertoire of κ−/− mice born to κ−/− mothers. We studied the repertoires in 8-wk-old mice that had already recovered roughly 50% of the control Cκ-specific response and in 26- and 52-wk-old mice which displayed a full proliferative response since 3 and 9 mo, respectively. A typical Immunoscope profile consists of only the public SADNYEQ sequence, which was readily detected by direct sequencing of PCR products (Table II). Repertoire analyses conducted on 26-wk-old mice which displayed a full proliferative response and in 52-wk-old mice. Moreover, among 45 Vβ6-Jβ1.4 bacterial insert sequences of 9-aa-long CDR3 from three individuals, we never observed the public SIGGSNERL sequence (Table III). Nevertheless, it should be noted that in most 8- and 26-wk-old κ−/− mice born to κ−/− mothers, a SRTGSNERL sequence was detected (Table III, in 12 of 81 sequences). Analysis of bacterial cloning of Vβ6 rearrangements of 26-wk-old κ−/− mice born to κ−/− mothers, which display a dominant Immunoscope profile for the CDR3 of 9 aa (Fig. 4, panels 8 and 11), revealed the reappearance of the public SIGGSNERL sequence in two mice and a very conserved sequence (SIGGANERL) in a third one (Table III, right). Here again the canonical SIGGSNERL sequence was easily retrieved in all of the 52-wk-old mice.

Taken together, analyses of Vβ2 and Vβ6 rearrangements reveal that maternal Igκ induce long-lasting but reversible modifications in the Cκ-specific CD4+ T cell repertoire of offspring subjected to Igκ through physiological maternal transfer.

Finally, Immunoscope profiles of the Vβ13 rearrangement displayed the 9-aa-long CDR3 peak (Fig. 4, panels 13–18), and direct sequencing of Vβ13-Jβ2.3 PCR products conducted at any age revealed that the two public CDR3 sequences were identical with those described in regular κ−/− mice (Tables I and IV).

Discussion

The present report focuses on the nature of recovering the T cell repertoire reemerging after breakdown of tolerance. To this end, the Cκ-specific CD4+ T cell response was followed in H-2b κ−/− C57BL/6 mice immunized with a single dose of Cκ peptide. A typical analysis of one mouse is represented for each age and is representative of six individual mice.

FIGURE 4. CDR3β size distributions of Vβ2-, Vβ6-, and Vβ13-Cβ rearrangements of Cκ peptide immunized 8-, 26-, and 52-wk-old κ−/− mice born to κ−/− mothers. Immunoscope analyses have been conducted as in Fig. 3, but were done with LNC from Cκ peptide-primed mice restimulated in vitro with only the Cκ peptide. A typical analysis of one mouse is presented for each age and is representative of six individual mice.
offspring born to $\kappa^{+/-}$ mothers. In this system, maternal Ig are available for the progeny under physiological conditions from fetal life until weaning in the absence of manipulated Ig concentrations and intentional transfer. We show that maternal Ig supply induces a profound but reversible state of tolerance in the CD4$^+$ T cell compartment. Moreover, after having determined that the C$k$-specific CD4$^+$ T cell response in mice that have never seen $\kappa$ light chains is characterized by three public V$\beta$ rearrangements, we demonstrate that after tolerance breakdown each of the public rearrangements is characterized by a specific delay of reemergence. At 1 yr of age the public T cell repertoire is indistinguishable from that of regular $\kappa^{+/-}$ mice, suggesting its resilient nature.

Table III. V$\beta$-J$\beta$1.4 CDR3$k$ amino acid sequences from C$k$ peptide-immunized 8- to 52-wk-old $\kappa^{+/-}$ mice born to $\kappa^{+/-}$ mothers

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* Sequencing of V$\beta$-J$\beta$1.4 PCR products. See legend to Table 2.
Ck-specific CD4+ T cell tolerance in k−/− mice born to k+/− mothers

It has been known for a long time that large quantities of intact maternal Ig reach the offspring to ensure passive protection of newborns against pathogens while their immune system is not fully competent (for review see Ref. 40). It is only more recently that research has focused on T cells specific for Ig-derived peptides in the progeny (20, 22, 41, 42).

Our data show that Ck-specific CD4+ T cells are tolerant in k−/− mice born to k+/− mothers. This Ck-specific CD4+ T cell tolerance is strong in 4- to 5 wk-old pups but progressively vanishes. Indeed, 50% of the control response is recovered by 8 wk of age and normal proliferative responses are reached by 12 wk. Interestingly, the breakdown of Ck-specific CD4+ T cell tolerance correlates in time with the absence of Ck peptide presentation by class II cells of k−/− mice born to k+/− mothers. These data are in agreement with other studies that show the need for Ag persistence in the maintenance of T cell tolerance, even when soluble Ag was administered from early ontogeny (20, 22, 43).

In a previous study (22) using several crosses and foster nursing systems, we showed that colostrum/milk-transmitted Igk were administered from early ontogeny (20, 22, 43). This has been shown to be more efficient than T cell fusion in identifying public compared with private clones, the latter being more diverse in terms of TCR repertoire, utilize one or very few public clonal T cell expansions of in vitro restimulated LNC were analyzed (22, 41, 42). That research has focused on T cells specific for Ig-derived peptides in newborns against pathogens while their immune system is not ready for the emergence of a defined Ag leading to various primary immune responses. Indeed, no Immunoscope peak of the Vβ6-Jβ1.4 rearrangement was detectable from any k−/− mouse born to k+/− mothers, suggesting that the T cell precursors expressing the Vβ6-Jβ1.4 sequence are absent, anergized, or that their frequency is too low to efficiently participate in the Ck-specific T cell response. Finally, although an Immunoscope peak corresponding to the Vβ6-Jβ1.4 rearrangement is strongly increased in 8-wk-old k−/− mice born to k+/− mothers in response to the Ck peptide, we were unable to detect a dominant CDR3β sequence. Moreover, of 61 molecular sequences obtained from five individual mice (three of them shown in Table III), none displayed the recurrent SIGGSNERL CDR3β sequence. This suggests that other Ck-specific CD4+ T cells using the same Vβ6-Jβ1.4 rearrangement and an identical CDR3β size developed, and that the T cell precursors bearing the SIGGSNERL CDR3β were absent or highly underrepresented. It has already been shown in other antigenic systems that restricted Vβ, Jβ, and CDR3β size may be used in response to a defined Ag leading to various primary CDR3β sequences (46, 47). However, it is possible that many of the Vβ6-Jβ1.4 CDR3β sequences from 8-wk-old k−/− mice born to k+/− mothers specifically participate in the Ck peptide response and correspond to private or public rearrangements, which may be underestimated in regular k−/− mice because they are dwarfed by the dominant SIGGSNERL CDR3β sequence. One possible candidate would be the SRTGSGNL CDR3β sequence which is found in molecular clones of five of six k−/− mice born to k+/− mothers (Table III, in 12 of 81 sequences) and in very few bacterial colonies from regular k−/− mice (data not shown). This sequence could be public in k−/− mice born to either k+/− or k−/− mothers.
but in the latter they cannot be detected by direct sequencing because of the strong dominance of the SIGGNSERL sequence which emerged in response to the Ck peptide.

When Ck-specific CD4+ T cells have fully escaped from tolerance (at 26 wk of age), the Immunoscope profiles obtained in response to the Ck peptide were closer to the ones obtained from regular κ−/− mice. Indeed, the peak corresponding to Vβ2-Jβ2.7, with a CDR3 of 7 aa, reappeared. Nevertheless, in contrast to regular κ−/− mice, the dominant SADNYEQ CDR3β sequence cannot be obtained by direct sequencing of Vβ2-Jβ2.7 PCR products but was found in bacterial clones from only one κ−/− mouse born to a κ+/− mother. Another mouse developed a similar SGDNYEQ sequence. This rearrangement seems to slowly reappear, but its frequency is still very low in most animals when compared with regular κ−/− mice. Similar conclusions could be drawn from Vβ6-Jβ1.4 sequences studied in CD4+ T cell responses of 26-wk-old κ−/− mice born to κ+/− mothers. Here again, the public CDR3β sequence (SIGGNSERL) is only found in two individuals, while a third animal displayed a related SIGGANERL sequence. Finally, at 52 wk of age, the three canonical Vβ rearrangements could be readily detected by direct sequencing of the corresponding Vβ-β PCR products in five of five mice tested, suggesting that their frequency reached that of mice which had never seen the κ light chain.

What are the constraints to which the recovering Ck-specific T cells are subjected?

Although each of the three Vβ public rearrangements is involved in the recognition of a single antigenic peptide, they may recognize distinct epitopes of the same peptide-MHC complex. Accordingly, each individual clone may be submitted to independent tolerance mechanisms and to distinct selecting ligands even if positive selection involves a few sets of peptides (48,49).

Using various experimental strategies, we have not been able until now to define a potential active process of suppression similar to that we had described in the tolerance of Ck-specific CD8+ T cells (22). Similarly, we cannot exclude the possibility that Ck-specific CD4+ T cells are anergized in κ−/− mice born to κ+/− mothers, although we were unable to reverse the Ck-specific T cell response of the tolerantized mice when co cultured with Ck peptide and various concentrations of IL-2 (data not shown). Because maintenance of suppression and anergy are known to require permanent presentation of the Ag (24), meaning of κ−/− mice born to κ+/− mothers may induce faster reversion of Ck-specific CD4+ T cell tolerance than that occurring after deletion. We have shown that maternal exogenous Igκ are efficiently processed and that the Ck-derived peptides are presented by thymic cells of κ−/− mice born to κ+/− mothers. It is therefore conceivable that this mode of presentation leads to deletion of developing Ck-specific CD4+ T cells. However, we failed to obtain conclusive answers to this point inasmuch as thymocyte introduced large variations into the Ck-specific T cell responses of regular mice κ−/−.

Alternatively, the delay of reemergence could be dependent on mechanistic constraints. Indeed Vβ13 rearrangement which is devoid of N additions could emerge faster than Vβ6 and Vβ2 rearrangements which include a fewer number of N additions (data not shown). Finally, the rate of division of each of these clones may influence the kinetics of their reemergence.

Whatever the responsible mechanisms may be that are responsible for the kinetics of reappearance of the three public clones, our study is the first to demonstrate that a T cell repertoire that has been profoundly disturbed for several months returns to equilibrium provided a sufficient period of time is allowed to pass.

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References


