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

Mathias Faure, Sébastien Calbo, Jean Kanellopoulos, Anne-Marie Drapier, Pierre-André Cazenave and Dominique Rueff-Juy

*J Immunol* 1999; 163:6511-6519; ;
http://www.jimmunol.org/content/163/12/6511

---

**References**

This article cites 45 articles, 23 of which you can access for free at: http://www.jimmunol.org/content/163/12/6511.full#ref-list-1

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

**Permissions**

Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
Tolerance to Maternal Immunoglobulins: Resilience of the Specific T Cell Repertoire in Spite of Long-Lasting Perturbations

Mathias Faure,²* Sébastien Calbo,† Jean Kanellopoulos,‡ Anne-Marie Drapier,* Pierre-André Cazenave,* and Dominique Rueff-Juy³*

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 κ light chain knock-out (κ⁻/⁻) mice born to κ⁺/⁺ mothers. These cells, which were in contact with maternal κ⁺ Igs from early ontogeny until weaning, were strongly tolerized. Tolerance was reversible and waned with the disappearance of peptide Ck₁₃₄–₁₄₈ presentation in lymphoid organs, including the thymus. Whereas three specific VB-Jβ 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 VB-Jβ 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.

Most circulating T cells are generated in the thymus and express heterodimeric TCR consisting of α- and β-chains (1). The recognition structure of this heterodimer is encoded by a series of stochastic rearrangements between variable (Vα), joining (Jα) and Vβ, diversity (Dβ), and Jβ gene segments (2). Imprecise junctions and nucleotide additions increase the diversity emerging from this process (3). The loop formed by the α and β junctional regions, or hypervariable complementarity determining region 3 (CDR3), interacts directly with the peptide/MHC complex (1, 4). This interaction is crucial not only for ensuring T cell specificity but also for shaping the T cell repertoire. Indeed, the potential repertoire generated during T cell development is characterized by a huge diversity of TCR displaying various ranges of avidities for self peptide/MHC complexes and accordingly must be submitted to positive and negative processes of selection (5–7).

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” components (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 Ab 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.

*Unité d’Immunochimie Analytique (URA Centre National de la Recherche Scientifique 1961 and Université Pierre et Marie Curie), and † Unité de Biologie Moléculaire du Gène, Institut Pasteur, Paris, France

Received for publication August 13, 1999. Accepted for publication October 7, 1999.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¹ This work was supported by the Ministère de l’Education Nationale, de la Recherche et de la Technologie, and by the Pasteur-Weissmann Fondation (to M.F.). S.C. was a fellow of the Ministère de l’Éducation Nationale, de la Recherche et de la Technologie, and † Unité de Biologie Moléculaire du Gène, Université Pierre et Marie Curie, and † Unite de Biologie Moléculaire du Gène, Institut Pasteur, 75724 Paris cedex 15, France. E-mail address: rueffjuy@pasteur.fr

Address correspondence and reprint requests to Dr. Dominique Rueff-Juy, Unité d’Immunochimie Analytique, Institut Pasteur, 75724 Paris cedex 15, France. E-mail address: rueffjuy@pasteur.fr

² Abbreviations used in this paper: CDR3, hypervariable complementarity determining region 3; Ck, κ light chain constant region; Igs, κ positive Igs; Cx peptide, peptide Cx₁₃₄–₁₄₈; κ⁻/⁻ mice, κ⁻/⁻ mice born to κ⁻/⁻ mothers.
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 k light chain constant


verse C

D

molecules. The comparison of the repertoires of Ck-specific CD4⁺ T cells of regular k⁻/⁻ mice and those of k⁻/⁻ mice born to k⁺/⁻ mothers was conducted making use of the Immunoscope method described by Pannetier et al. (37) and sequence analysis. Our data show that the CD4⁺ Ck-specific T cell response in H-2² regular k⁻/⁻ mice is characterized by the expression of three distinct public Vβ rearrangements. A genetic analysis of the Vβ repertoire of k⁻/⁻ mice born to k⁺/⁻ mothers clearly indicates that maternal Igk induce long-lasting but reversible modifications in the T cell repertoire, highlighting the resilient nature of this repertoire.

Materials and Methods

Mice

H-2² k⁻/⁻ mice were used in this study and in our previous study (36). Briefly, H-2² k⁻/⁻ mice were obtained using k-deficient 129 mice (k⁺/⁻, H-2²) generated by targeted mutation in the Ck gene from 129/Sv embryonic stem cells (38) as follows: mice with the k⁺/⁻, H-2² phenotype were selected in the F₂ generation obtained by crossing between (BALB/c × 129 k⁺/⁻)F1 mice. These individuals were bred by brother/sister crossings, and mice belonging to the seventh generation were used. k⁻/⁻ mice born to k⁺/⁻ mothers were identified by FACS analysis at 3–4 wk of age, as described previously (9).

Proliferation assays

Mice (4–26 wk old, as indicated in figure and table legends) were immunized in the hind footpads with 10 μg of Ck peptide (sequence CFFLNFYFPKDINNVK; Syntem, Nîmes, France) emulsified in CFA. Eight days after immunization, 5 × 10⁶ lymph node cells/well of 96-well culture plates were tested for their ability to proliferate in response to serial concentrations of k light chain (as described previously in Ref. 36). Ck peptide, or purified protein derivative of tuberculin (PPD), in synthetic HL-1 medium (BioWhittaker, Walkersville, MD) supplemented with 2 mM L-glutamine. When a single concentration is represented, it corresponds to a point from the exponential phase of proliferation. Cultures were pulsed with 1 μCi of [³H]thymidine (1 Ci = 37 GBq) for the last 13 h of a 4-day culture. Results are expressed as Δcpm (mean of triplicates and background).

In the kinetics assays, results are expressed as the percentage of individual response to k light chain in comparison with the one obtained in response to PPD as follows: 100 × (proliferation with k light chain−background)/proliferation with PPD−background.

T cell hybridomas and tissue localization of Ck peptide

T cell hybridomas M44-C2 and M67-C11 were previously described (36). The stimulation assays were performed with 10⁵ hybridoma cells which were cultured with irradiated nonpurified cells (extracted from the irradiated lymphoid tissues) from k⁻/⁻ mice born to k⁺/⁻ mothers, in 96-well tissue culture plates. After 24 h, secretion of IL-2 in supernatants was measured by proliferation of IL-2-dependent CTL-L2 cells after [³H]ThdR incorporation as previously described (36).

RNA extraction and cDNA synthesis

The Ck-specific public T cell repertoire was determined from individual H-2² k⁻/⁻ mice born to k⁺/⁻ mothers immunized with Ck peptide in CFA. mRNA from lymph node cells (LNC) stimulated for 4 days in 2 ml at 2.5 × 10⁶ cells/ml with 25 μg/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 after depletion of CD8⁺ T cells using the biotinylated anti-CD8 14.5 Ab (39) and streptavidin M-280 Dynabeads (N-0121; 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 Ck-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 cells. The technique consists of two steps. In the first one, PCR conducted with specific Vβ and Cb oligonucleotides amplify TCR with specific Vβ sequences but different CDR3 lengths. In the second step, Vβ-Cb PCR products are submitted to run-off reactions with different labeled Jβ 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 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 μl on 1/40 of the cDNA using 2 U of Taq polymerase (Eurogentec, Seraing, Belgium) in the supplier’s buffer with a Vβ-specific sense primer and the Cβ4 antisense primer as previously described (12). To cover the full repertoire, PCR were conducted using the primers specific for the 23 functional Vβ 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 Cβ oligonucleotide (Cβ5') 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-2² k⁻/⁻ mice after restimulation with Ck peptide only, but not after PPD restimulation, were subjected to run-off reactions using all 12 Jβ-labeled specific primers.

Sequences

An aliquot of each of the specific Vβ-Cβ4 PCR products (Vβ2-, Vβ6-, and Vβ13-Cβ4) was amplified by 25 cycles with their specific Vβ and Jβ primers and was submitted to an elongation step at 72°C for 10 min. Three different sequence analysis strategies were conducted. 1) Direct sequencing of PCR products: 5 μl of the Vβ-Jβ amplifications were treated with 0.25 μl of exonuclease at 10 U/μl (Amersham, Orsay, France) and with 0.25 μl shrimp alkaline phosphatase (Amersham) at 1 U/μl for 40 min at 37°C and then for 20 min at 80°C. Sequences were then conducted with the corresponding Vβ primers using the Big Dye Terminator kit (Perkin-Elmer Applied Biosystems, 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 μl of the Vβ-Jβ 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 μl distilled water, and the inserts were directly PCR-amplified in 40 μl 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 Vβ2-Jβ2.7 rearrangements of k⁺ mice born to k⁻/⁻ mothers. Briefly, following restriction 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

Downloaded from http://www.jimmunol.org/ by guest on April 20, 2017
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.

The Journal of Immunology

6513

Results

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

To determine the influence of maternal Ig on the T cell progeny, we assessed the Ck-specific T cell response of Ck−/− mice born to Ck+/− mothers. In this situation, mice were exposed to large amounts of Igk from fetal life to weaning. The proliferative activity of both κ light chain- and Ck peptide-restimulated LNC of Ck−/− mice born to Ck+/− mothers immunized at 4 wk of age with Ck peptide is totally abolished when compared with that of age-matched immunized regular Ck−/− mice and is similar to that of Ck+/− 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 κ 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, Ck−/− mice born to Ck+/− 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 tolerized mice using Ck-specific T cell hybridomas. Fig. 2 shows that maternal Igk-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

FIGURE 1. Ck-specific T cell proliferative responses. A, Four wk-old H-2d Ck+/− regular mice (■), Ck−/− mice born to Ck−/− mothers (□), or Ck−/− 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. The CD4+ T cell response from Ck−/− mice born to Ck+/− mothers (Ο, thick curve) and Ck−/− mice (Ο, 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 κ light chain-specific response in comparison to the PPD-specific response (κ 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 Ck−/− mice was 61 ± 4%.

FIGURE 2. Kinetics of presentation of maternal Igk-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 Ck−/− mice born to Ck+/− 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 105 CTLL for 3 days. Results are expressed as average cpm of triplicate culture ± SD of [3H]TdR incorporation added for the last 6 h of CTLL 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 Ck−/− pups born to regular Ck−/− mice induced less than 500 cpm of CTLL proliferation.
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 Ck-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 Ck peptide presentation.

Characterization of the Ck-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 Ck-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 Ck peptide in CFA were restimulated in vitro with Ck 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 Ck 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-restimulated LNC from Ck peptide-primed κ−/− mice (panels 13–18). Moreover, the recurrent Ck-specific T cell rearrangements were elicited against the naturally processed Ck peptide because identical Immunoscope profiles were observed whether LNC were restimulated in vitro with Ck peptide or with native κ light chain (panels 7–12). Although Ck peptide used for immunization and in vitro restimulation is synthetic, these data suggest that it contains only epitopes expressed by the naturally processed Ck 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 (SRTGNSNERL) 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 Ck 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 Ck 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 Ck peptide-primed regular κ−/− mice. Eight-week-old mice were immunized with Ck peptide in CFA. LNC were harvested 8 days later and restimulated in vitro with Ck 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 Ck 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.


### Table 1. Public CDR3β amino acid sequences from Ck-immunized 8- to 52-wk-old regular κ−/− mice in response to Ck peptide or κ light chain

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Vβ2-Jβ2.7</th>
<th>Vβ6-Jβ1.4</th>
<th>Vβ13-Jβ2.3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SADNYEQ SIGGSNERL SFAGRAETL</td>
<td>7</td>
<td>SADNYEQ SIGGSNERL SFAGRAETL</td>
</tr>
<tr>
<td>2</td>
<td>SADNYEQ SIGGSNERL SFAGRAETL</td>
<td>8</td>
<td>SADNYEQ SIGGSNERL SWGGAETL</td>
</tr>
<tr>
<td>3</td>
<td>SADNYEQ SIGGSNERL SWGGAETL</td>
<td>9</td>
<td>SADNYEQ SIGGSNERL SFAGRAETL</td>
</tr>
<tr>
<td>4</td>
<td>SADNYEQ SIGGSNERL SFAGRAETL</td>
<td>10</td>
<td>SADNYEQ SIGGSNERL SWGGAETL</td>
</tr>
<tr>
<td>L1</td>
<td>SADNYEQ SIGGSNERL SFAGRAETL</td>
<td>1</td>
<td>SADNYEQ SIGGSNERL SFAGRAETL</td>
</tr>
<tr>
<td>L3</td>
<td>SADNYEQ SIGGSNERL SWGGAETL</td>
<td>3</td>
<td>SADNYEQ SIGGSNERL SFAGRAETL</td>
</tr>
</tbody>
</table>

* L1 and L3 correspond to Ck peptide-immunized mice no. 1 and 3 from which LNC were restimulated in vitro with κ light chain. Sequences are read from direct sequencing of Vβ-Jβ PCR products.

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

To determine the influence of maternal Igκ on the developing T cell repertoire of the offspring, we analyzed the functional 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 Ck-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 analysis of one mouse per age group, representative of six others, is shown in Fig. 4, and individual sequence analyses for each rearrangement are reported in Tables II, III, and IV.

Analysis of the Vβ2 rearrangement shows that the typical expansion of a 7-aa peak found in regular κ−/− mice (Fig. 3, panels 1 and 2) has completely disappeared in 8-wk-old κ−/− mice born to κ+/− mothers (Fig. 4, panels 1 and 4). Furthermore, the SADNYEQ public sequence or any homologous sequence was never found in the 18 sequences obtained from bacterial colonies containing the cloned Vβ2 7-aa-long rearrangement of one mouse (Table II). Repertoire analyses conducted on 26-wk-old κ−/− mice revealed a slight height increase of the peak corresponding to the public Vβ2 rearrangement (Fig. 4, panels 2 and 5). CDR3 sequence of Vβ2-Jβ2.7 inserts from bacterial colonies showed that the public SADNYEQ sequence was found only in one 26-wk-old mouse and a related SGDNYEQ sequence was found only in one other mouse. Finally, Fig. 4 (panel 6) shows that the typical profile of 7-aa-long Vβ2-Jβ2.7 CDR3 was retrieved at 52 wk of age and consists of only the public SADNYEQ sequence, which was readily detected by direct sequencing of PCR products (Table II).

Although Immunoscope profiles of the Vβ6 rearrangement were similar in both 8-wk-old regular κ−/− mice (Fig. 3, panels 3 and 4) and κ−/− mice born to κ+/− mothers (Fig. 4, panels 7 and 10), no sequence was readable after direct sequencing of PCR products in this last group. 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 SRTGNERL 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 (SIGGANGERL) 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 Ck-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 Ck-specific CD4+ T cell response was followed in H-2b κ−/−

**FIGURE 4.** CDR3β size distributions of Vβ2-, Vβ6-, and Vβ13-Cβ rearrangements of Ck 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 Ck peptide-primed mice restimulated in vitro with only the Ck peptide. A typical analysis of one mouse is represented for each age and is representative of six individual mice.
a profound but reversible state of tolerance in the CD4
and intentional transfer. We show that maternal Ig supply induces
available for the progeny under physiological conditions from fetal
offspring born to κ−/− 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 Cκ-spe-
cific CD4+ T cell response in mice that have never seen κ light
chains is characterized by three public Vβ rearrangements, we
demonstrate that after tolerance breakdown each of the public re-
arrangements is characterized by a specific delay of reemergence.
At 1 yr of age the public T cell repertoire is indistinguishable from
that of regular κ−/− mice, suggesting its resilient nature.

The T cell repertoire following breakdown of tolerance

Table II. Vβ2-Jβ2.7 CDR3β amino acid sequences from Cκ peptide-immunized 8- to 52-wk-old κ−/− mice born to κ−/− mothers

<table>
<thead>
<tr>
<th>8-wk-old, Bacterial Cloning</th>
<th>26-wk-old, Bacterial Cloning</th>
<th>52-wk-old, Direct Sequencing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>Sequence</td>
<td>n</td>
</tr>
<tr>
<td>p1</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>p4</td>
<td>SDLTYESQ</td>
<td>4/18</td>
</tr>
<tr>
<td>p6</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Since CDR3 sequences could not be read by direct sequencing of Vβ2-Jβ2.7 PCR products, the PCR products corresponding in the CDR3 length of interest were cloned.

In this table, n = number of identical amino acid sequences found/total number of sequences.
Sequences read from direct sequencing of Vβ2-Jβ2.7 PCR products.

The T cell repertoire following breakdown of tolerance

Table III. Vβ6-Jβ1.4 CDR3β amino acid sequences from Cκ peptide-immunized 8- to 52-wk-old κ−/− mice born to κ−/− mothers

<table>
<thead>
<tr>
<th>8-wk-old, Bacterial Cloning</th>
<th>26-wk-old, Bacterial Cloning</th>
<th>52-wk-old, Direct Sequencing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>Sequence</td>
<td>n</td>
</tr>
<tr>
<td>p1</td>
<td>SIPGSNERL</td>
<td>2/15</td>
</tr>
<tr>
<td>p4</td>
<td>SRTGSNERL</td>
<td>2/16</td>
</tr>
<tr>
<td>p6</td>
<td>SIPGSNERL</td>
<td>2/14</td>
</tr>
</tbody>
</table>

Sequencing of Vβ6-Jβ1.4 PCR products. See legend to Table 2.
Ck-specific CD4+ T cell tolerance in κ−/− mice born to κ+/− 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 tolerated in κ−/− mice born to κ+/− 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 κ−/− mice born to κ+/− 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 are very efficient in inducing and maintaining tolerance in Ck-specific CD8+ T cells. In this paper, we present the first direct evidence that maternal Igk, most likely delivered via the oral route, are efficiently presented by thymic cells to thymocytes. Igk might reach the corticomедullary junction of the thymus through the bloodstream, where thymic APC process them. Alternatively, peripheral APC which have already processed Igk could migrate to the thymus to present κ light chain-derived peptides.

Ck-specific T cell public repertoire in regular κ−/− mice

To study the reemergence of the Ck-specific CD4+ T cell repertoire after tolerance breakdown, we first had to identify the public T cell response to Ck peptide in regular κ−/− mice. To this end, clonal T cell expansions of in vitro restimulated LNC were analyzed by the Immunoscope technique. T cell responses, which are diverse in terms of TCR repertoire, utilize one or very few public clones, and several private clones. The Immunoscope technique has been shown to be more efficient than T cell fusion in identifying public compared with private clones, the latter being more readily derived by hybridoma technology or T cell cloning (11, 12, 44). Making use of the Immunoscope technique, we constantly found the emergence of three distinct recurrent Vβ-Jβ rearrangements: Vβ2-Jβ2.7, Vβ6-Jβ1.4, and Vβ13-Jβ2.3. These rearrangements were detected after in vitro κ light chain-restimulation of Ck peptide-primed LNC. This strongly suggests that the three recurrent rearrangements are used in the Ck-specific T cell response to the physiologically processed Ck peptide. In each mouse, the SADNYEQ CDR3 sequence was readily detectable from direct sequencing of the Vβ2-Jβ2.7 PCR product, suggesting a very high frequency of this particular Vβ clonotypic rearrangement in response to the Ck peptide. Similar conclusions can be drawn from the analysis of the Vβ6-Jβ1.4 rearrangement, which showed the recurrent usage of the same CDR3β loops (SIGGSNERL) by each mouse. We also found from direct Vβ13-Jβ2.3 PCR products the emergence of two very “synonymous” (45) CDR3β loops (SFAGRAETL, SWGGRAETL), which diverge from each other by conservative substitutions of two residues at positions 96 and 97 of the CDR3β: F or W (both aromatic and hydrophobic) at position 96, and A or G (both small and aliphatic) at position 97.

Although CDR3 amino acid sequences of each public rearrangement are highly conserved in all mice, variations in codon usage were found in N diversity regions (data not shown), strongly suggesting that TCRs involved in public responses are highly selected for.

Recovery of the Ck-specific T cell repertoire in κ−/− mice born to κ+/− mothers

First, we analyzed the newly reemerging T cell repertoire of 8-wk-old κ−/− mice born to κ+/− mothers, of which only 50% of the Ck-specific T cell proliferative response is recovered. Interestingly, only the Vβ13-Jβ2.3 public and synonymous associated rearrangements are detected and participate in the Ck-specific CD4+ T cell response. Indeed, no Immunoscope peak of the Vβ2-Jβ2.7 rearrangement was detectable from any κ−/− mouse born to κ+/− mothers, suggesting that the T cell precursors expressing the Vβ2-Jβ2.7 rearrangement with the public CDR3 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 rearrangements is strongly increased in 8-wk-old κ−/− mice born to κ+/− mothers, 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 CDR3 sequence. This suggests that other Ck-specific CD4+ T cells using the same Vβ6-Jβ1.4 rearrangement and an identical CDR3 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 CDR3 sequences from 8-wk-old κ−/− mice born to κ+/− mothers specifically participate in the Ck peptide response and correspond to private or public rearrangements, which may be underestimated in regular κ−/− mice because they are dwarfed by the dominant SIGGSNERL CDR3β sequence. One possible candidate would be the SRTGSGRL sequence which is found in molecular clones of five of six κ−/− mice born to κ+/− mothers (Table III, in 12 of 81 sequences) and in very few bacterial colonies from regular κ−/− mice (data not shown). This sequence could be public in κ−/− mice born to either κ+/− or κ−/− mothers.

---

Table IV. Vβ13-Jβ2.3 CDR3 amino acid sequences from Ck peptide immunized 8- to 52-wk-old κ−/− mice born to κ+/− mothers

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Sequence</th>
<th>Mouse</th>
<th>Sequence</th>
<th>Mouse</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>p1</td>
<td>SWGGRAETL</td>
<td>p8</td>
<td>SWGGRAETL</td>
<td>YS1</td>
<td>SWGGRAETL</td>
</tr>
<tr>
<td>p4</td>
<td>SFAGRAETL</td>
<td>p9</td>
<td>SFAGRAETL</td>
<td>YS2</td>
<td>SWGGRAETL</td>
</tr>
<tr>
<td>p6</td>
<td>SWGGRAETL</td>
<td>p12</td>
<td>SWGGRAETL</td>
<td>YS3</td>
<td>SFAGRAETL</td>
</tr>
</tbody>
</table>

* Sequences are obtained from direct sequencing of Vβ13-Jβ2.3 PCR products.
but in the latter they cannot be detected by direct sequencing because of the strong dominance of the SIGGSNERL 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 Vb2-Jb2.7, with a CDR3 of 7 aa, reemerged. Nevertheless, in contrast to regular κ⁻/⁻ mice, the dominant SADNYEQ CDR3β sequence cannot be obtained by direct sequencing of Vb2-Jb2.7 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 Vb6-Jb1.4 sequences studied in CD4⁺ T cell responses of 26-wk-old κ⁻/⁻ mice born to κ⁺/⁺ mothers. Here again, the public CDR3β sequence (SIGGSNERL) is only found in two individuals, while a third animal displayed a related SIGGANERL sequence. Finally, at 52 wk of age, the three canonical Vb rearrangements could be readily detected by direct sequencing of the corresponding Vb-Jb 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 contraints to which the recovering Ck-specific T cells are subjected?

Although each of the three Vb 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 which 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 tolerated mice when cocultured 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 thymectomy 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 Vb13 rearrangement which is devoid of N additions could emerge faster than Vb6 and Vb2 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.

Acknowledgments

We thank Armanda Casrouge and Sophie Dalle for technical advice and assistance, Prof. Gilles Marchal for his kind gift of PPD, Dr. Pierre Boudinot and Prof. Pierre Sanchez for helpful discussions, and Prof. Emmett Johnson and Dr. David Ojcius for critical reading of the manuscript.

References


