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*J Immunol* 2008; 181:6757-6769; doi: 10.4049/jimmunol.181.10.6757
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Reversible Blockade of Thymic Output: An Inherent Part of TLR Ligand-Mediated Immune Response

Thomas Démoulins,‡ Ali Abdallah,‡ Nadia Kettaf,‡ Marie-Laurence Baron,‡ Casimiro Gerarduzzi,* Dominique Gauchat,* Sophie Gratton,* and Rafick-Pierre Sékaly*‡§¶

TLRs constitute a first set of sensors that detect viral nucleic acids including dsRNA which triggers TLR3. We report the early, direct, and detrimental effect of polyinosine-polycytidilic acid treatment on T cell development. Inhibition of thymopoiesis was targeted to several thymocyte subpopulations. First, both a blockade of the double negative (DN1-DN2) transition and a severe down-regulation of DN3-DN4 thymocyte proliferation were observed. In addition, an important decrease in the absolute numbers of double-positive thymocytes, concomitant with an increase in frequencies of apoptotic cells in this population were shown. This inhibition of thymopoiesis resulted in a reduced thymic output, as evidenced by a drop of the absolute numbers of naive T cells and TCR excision circles levels. The decrease in thymic cellularity and defects in thymic development were severely reduced, but not completely abolished in IFN-α/βR−/− mice, showing a direct contribution of type I IFNs, known to be massively up-regulated in viral infections, to the inhibition of T cell development. Strikingly, the TCR repertoire in treated mice was biased toward shorter CDR3 lengths as a result of a decreased expression of TdT and Rag2. However, thymic integrity remained intact since thymopoiesis was restored both quantitatively and qualitatively 14 days after the cessation of polyinosine-polycytidilic acid treatment. These results demonstrate a novel immunomodulatory role for virally encoded TLR ligands and RNA sensors; they further illustrate the diversity of mechanisms that viruses use to interfere with the development of a pathogen-specific immune responses. The Journal of Immunology, 2008, 181: 6757–6769.

oll-like receptors are major components of the innate immune system in that they are key sensors of pathogens, including viral nucleic acids. In mice, 12 different TLRs have been identified, including TLR3; TLR3 recognizes dsRNA and signals downstream through Toll/IL-1R domain-containing adaptor-inducing IFN-β to lead to the production of high levels of type I IFNs that are a hallmark of viral infections (1). Type I IFNs are a heterogeneous group of proteins that include IFN-α, IFN-β, and the less extensively studied IFN-ε, IFN-κ, IFN-ω, IFN-δ, and IFN-τ (2). IFN-α/β are inducible cytokines that play a direct and crucial role in the orchestration of the early innate response to viral infections. This innate immune response to viral infections mediated by type I IFNs is characterized by: 1) an antiviral activity on target cells and the induction of apoptosis of infected cells (3, 4), 2) antiproliferative and immunomodulatory activities (5–7), and 3) the up-regulation of MHC class I expression by dendritic cells which promote presentation of virally encoded epitopes to CTLs (8).

Recently, the impact of type I IFNs during the acute phase of viral infections on cellular immune responses to these pathogens has been reported. First, they contribute to enhance T lymphocyte clonal expansion, thereby increasing the size of the memory T cell pool (9–15). They also protect activated naive T cells from death, which impacts on the magnitude of their homeostatic proliferation and survival (16). Strikingly, the outcomes of type I IFNs described so far on thymocytes are mostly deleterious and quite different from the prosurvival properties reported in mature peripheral T lymphocytes. First, a strong decrease of thymic cellularity, mostly characterized by a severe fall in the number of double-positive (DP) cells, was evidenced in newborn mice treated with an active human IFN-α2/α1 hybrid molecule (17). In addition, MHC class I up-regulation mediated by IFN-α was demonstrated in HIV-1-infected fetal thymic organ cultures (FTOCs) (18, 19). Finally, exogenously added IFN-α reversibly interferes with the development of human thymic CD34+CD1a− precursors into T cells, raising the possibility that type I IFNs could alter the development of the T cell lineage (20).

In this report, we have investigated the detrimental impact of polyinosine-polycytidilic acid (poly(I:C)) treatment on thymopoiesis. Mice were treated i.p. with poly(I:C), a strong type I IFNs inducer that mimics virally encoded dsRNA; poly(I:C) is a TLR3 ligand (21) (22) and also binds the RNA sensor RIG-I (for a review, see Ref. 23); we analyzed the impact of this treatment on several steps of cell development. We have also determined the

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Received for publication July 2, 2007. Accepted for publication September 6, 2008.

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1 This work was supported by grants to R.-P.S. from the Canadian Institutes of Health Research (CIHR) and from the Canadian Network for Vaccine and Immunotherapeutics. R.-P.S. is the Canada Research Chair in Human Immunology.
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impact of poly(I:C) treatment on qualitative and quantitative features of exiting thymocytes and naïve T cells. We showed that in the early days following initiation of poly(I:C) treatment, the cellular immune response is characterized by an arrest of thymopoiesis, leading to both a reduced thymic output and a restriction of the de novo-produced TCR repertoire.

Materials and Methods

Mice and treatment

Four- to 6-wk-old female C57BL/6 mice were purchased from the Charles River Laboratory. Four- to 6-wk-old female 129Sv/Ev mice were purchased from Taconic Farms. Four- to 6-wk-old female type I IFN receptor knockout (IFN-α/β−/−, 129Sv/Ev genetic background) were purchased from B&K Universal Limited (24). Four- to 6-wk-old female Rag-2p-GFP mice were a generous gift from M. Nussenzweig (The Rockefeller University, New York, NY). Mice were treated daily for variable durations i.p. with poly(I:C) (InvivoGen) or PBS. Poly(I:C) was certified LPS free by the manufacturer. Sacrifice occurred 3 h after the last injection. In other experiments designed to examine the recovery from poly(I:C) treatment, mice were injected 3 days with poly(I:C) and killed 7, 14, 21, and 28 days after the final injection. The design of each experiment contained six mice per group per time point, except in some poly(I:C) groups where 10–15% of the final injection. The design of each experiment contained six mice per group per time point, except in some poly(I:C) groups where 10–15% of the mice died during the treatment. Animal studies were approved by the local ethical committee (Comité Institutionnel de Protection des Animaux).

Isolation of organs and cell suspension

After sacrifice, thymus and mesenteric lymph nodes (mLNs) were isolated, mechanically disrupted, treated for 10 min with DNase (Sigma-Aldrich), and collagenase-D (Roche), ACK solution (NH4Cl KHCO3 EDTA), and prepared as single-cell suspensions. Enzymatic treatments were terminated by the addition of an equal volume of FCS, followed by washing the cells in PBS plus 10% FCS.

Flow cytometry and cell sorting

For the detection of cell surface markers, 2 × 10^6 cells were incubated with the appropriate Abs for 30 min on ice, followed by washing. Live events were collected based on forward and side scatter profiles on an LSRII flow cytometer (BD Biosciences), and the data were acquired and analyzed with FACS Diva software (BD Biosciences). Abs were purchased from BD Pharmingen and included CD44-PE, CD25-allophycocyanin-Cy7, CD8-PE-Cy7, CD3-Alexa Fluor 700, CD4-PE-Cy7, CD8-allophycocyanin-Cy7, CD8-Cy5, CD45-PE-Cy7, B220-PE, CD69-PE, CD24-PE, and annexin V-FITC.

For staining with anti-BrdU, animals were injected i.p. twice with 1 mg of BrdU (Sigma-Aldrich) and the thymus was taken 1 h after the last injection. To specifically evaluate early thymocyte progenitors, we studied CD44 and CD25 expression in PerCP lineage-negative (lin−) cells from mice (BD Pharmingen). The lin− cells were defined as negative for lineage marker surface expression: CD11b (Mac-1), Ly-6G (Gr.1), CD45R/B220, and TER-119/erythroïd cells (Ly-76). Cells (2 × 10^6) were stained for cell surface markers, then fixed and permeabilized with paraformaldehyde/ Tween 20 and stained using the FITC-conjugated BrdU kit (BD Pharmingen) according to the manufacturer’s instructions. A minimum of 2 × 10^6 events in the live cell gated on CD25+ CD44− was accumulated for each sample.

For TCRβ repertoire experiments, thymic cells were stained with Abs CD3-Alexa Fluor 700, CD4-PE-Cy7, CD8-PE-Cy7, and CD8-allophycocyanin-Cy7 after mechanical dissociation of thymic tissue. The cells were then sorted with the FACS Aria cell sorter (BD Biosciences) and lysed in RLT buffer (QiaGen). Serologic analysis

Serum levels of IFN-α and IFN-β were determined in mice injected with 200 μg of poly(I:C) using an ELISA kit (PBL Biomedical Laboratories). Serum levels of corticosterone were determined after injection of 200 μg of poly(I:C), 100 μg of anti-TCRβ (HS7-597), or PBS using an ELISA kit (Neogen).

Quantification of TCR excision circles (TRECs)

Primers specific for the sjTCREs (Jo58 and Jo61), DJβ1 TRECS (DJβ1-Jβ1.1-DJβ1-Jβ1.6 and DJβ2-Jβ2.1–DJβ2-Jβ2.7), and the mouse CD4 gene were defined for mouse sequences from GenBank (accession numbers for the TCRβ6 locus: M64239, TCRβ locus: AE000663.4–5, and CD4: AC002597). Parallel quantification of each deletion circle along with the CD4 amplicon was performed for each sample using the LightCycler technology (Roche Diagnostics). Cells from mLNs were lysed in Tween 20 (0.05%), Nonidet P-40 (0.05%), and protease K (100 μg/ml) for 30 min at 56°C and then 15 min at 98°C. Multiplex PCR amplification was performed for the different TRECs along with CD4 in 100 μl (10-min initial denaturation at 95°C, 30 s at 95°C, 30 s at 60°C, 2 min at 72°C for 22 cycles) using outer 3/5’ primers pairs. PCR conditions in the LightCycler experiments, performed on one-twentieth of the initial PCR, were 1-min initial denaturation at 95°C, 1 s at 95°C, 10 s at 60°C, and 15 s at 72°C for 40 cycles; fluorescence measurements were performed at the end of the elongation steps. This highly sensitive quantitative PCR assay allows the detection of one copy of 10^6 cells for each DNA circle.

PCR analysis

cDNA was used as template in the semiquantitative PCR mix according to the manufacturer’s standard protocol (Invitrogen). The primer sequences were: TdT (sense), 5’-GAAGAGCAGAGGATGAGAAGA-3’; TdT (antisense), 5’-CATCATCTCTCTGTGTTG-3’; β-actin (sense), 5’-CTACACAGGCTTGTGA-3’; β-actin (antisense), 5’-CTGCTGCCAATAGTGAGA-3’; and helios (sense), 5’-ACCTCTCAGAGAACTTGTTGGG-3’; and helios (antisense), 5’-CCTGGGCCCTTTTGTTCCTTGT-3’ (25). PCR was conducted in a final volume of 50 μl containing 5 μl of the cDNA and 2 U of Taq polymerase in the buffer provided by the manufacturer. The cDNA was amplified for 35 cycles and PCR products were separated on an agarose gel.

TCRβ repertoire analysis

The 24 murine Vβ TCR chain-specific primers have been described elsewhere (26). The Cβ pool was also obtained from another study (27). In the present report, Vβ-β amplifications were performed with fluorochrome FAM, VIC, NED, or PET-labeled Cβ primer (Table D). Briefly, 200 ng of cDNA from each sample studied was subjected to 40 cycles of elongation for each of the 24 Vβ as follows: 30 s at 94°C, 30 s at 60°C, and 5 min at 72°C. After the final cycle, the mixture was heated for 10 min at 72°C. Positive PCR products were mixed in specific combinations to generate four distinct Vβ pools: P1 (Vβ2, Vβ4, Vβ8.3, Vβ10, Vβ13, and Vβ16), P2 (Vβ5.1, Vβ5.3, Vβ6.3, Vβ7.1, Vβ9, and Vβ20), P3 (Vβ7, Vβ8.2, Vβ9, Vβ11, Vβ12, and Vβ18) and P4 (Vβ1, Vβ3, Vβ5.2, Vβ8.1, Vβ14, and Vβ15) as shown in Table I. At least two independent rounds of PCRs were performed to ensure that negative signals were not artifactual. Electrophoresis was performed on a 3700 DNA sequencer (Applied Biosystems). CDR3 length and fluorescence intensity of PCR products were determined with GeneMapper software (Applied Biosystems). With unprimed and polyclonal T cell populations, the CDR3 size distribution of each Vβ-β-specific PCR product consisted of 6–10 Gaussian peaks separated by three nucleotides because they are derived from in-frame mRNAs. The area of each peak is proportional to the intensity

Table I. TCR repertoire experimental design

<table>
<thead>
<tr>
<th>Fluorochrome</th>
<th>Short Vβ</th>
<th>Long Vβ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vβ3 pool 1</td>
<td>Vβ10 (141)</td>
<td>Vβ4 (195)</td>
</tr>
<tr>
<td>VIC</td>
<td>Vβ16 (151)</td>
<td></td>
</tr>
<tr>
<td>NED</td>
<td>Vβ2 (164)</td>
<td>Vβ8.3 (220)</td>
</tr>
<tr>
<td>PET</td>
<td>Vβ13 (171)</td>
<td></td>
</tr>
<tr>
<td>Vβ3 pool 2</td>
<td>Vβ6 (149)</td>
<td>Vβ5.3 (205)</td>
</tr>
<tr>
<td>VIC</td>
<td>Vβ20 (155)</td>
<td></td>
</tr>
<tr>
<td>NED</td>
<td>Vβ19 (167)</td>
<td>Vβ5.1 (228)</td>
</tr>
<tr>
<td>PET</td>
<td>Vβ17 (173)</td>
<td></td>
</tr>
<tr>
<td>Vβ3 pool 3</td>
<td>Vβ9 (150)</td>
<td>Vβ12 (207)</td>
</tr>
<tr>
<td>VIC</td>
<td>Vβ11 (158)</td>
<td></td>
</tr>
<tr>
<td>NED</td>
<td>Vβ18 (175)</td>
<td>Vβ8.2 (234)</td>
</tr>
<tr>
<td>PET</td>
<td>Vβ17 (183)</td>
<td></td>
</tr>
<tr>
<td>Vβ3 pool 4</td>
<td>Vβ3 (156)</td>
<td>Vβ5.2 (219)</td>
</tr>
<tr>
<td>VIC</td>
<td>Vβ14 (161)</td>
<td></td>
</tr>
<tr>
<td>NED</td>
<td>Vβ1 (176)</td>
<td>Vβ8.1 (234)</td>
</tr>
<tr>
<td>PET</td>
<td>Vβ15 (180)</td>
<td></td>
</tr>
</tbody>
</table>

*Numbers in parentheses represent amplicons with a 10-aa CDR.*
of the fluorescent band and thus to the initial levels of each TCR transcript. Each peak for a given CDR3 size is likely to correspond to several different sequences.

**Statistical analysis**

Data are expressed as means $\pm$ SEM. Statistical significance of differences was determined by the paired two-tailed Student t test. Differences were considered statistically significant for $p < 0.05$. Statistical analyses were performed using Excel software (Microsoft).

**Results**

**Poly(I:C) treatment contributes directly to thymic involution**

In this study, we have investigated the impact of poly(I:C), on T cell development and thymic output. This model was chosen to dissociate the impact of type I IFN production from known deleterious consequences of viral replication on the homeostasis of the immune system (28–32). Our experimental protocol involved repeated daily injections of poly(I:C) over several days (33–35), since the impact of poly(I:C) impact is dependent on the dose and timing of its administration (36, 37) and its short half-life in vivo (38). First, C57BL/6 mice were injected i.p. daily for 3 days with 200 $\mu$g of poly(I:C) or PBS and were sacrificed 3 h after the last injection. Thymi were collected mechanically disrupted, treated for 10 min with collagenase D, and prepared as single-cell suspensions. Data represent thymic cellularity in function of poly(I:C) dose injected. Absolute counts were obtained by cell numeration. b. High levels of type I IFN protein in the serum of mice injected with poly(I:C). Two hundred micrograms of poly(I:C) or PBS was injected i.p. once (mice sacrificed 1, 3, 6, 12, and 24 h posttreatment) or daily for 3 days (mice sacrificed 1, 3, 6, and 12 h after the last injection) and IFN-α and β serum concentrations were determined by ELISA. Data presented are the means and SDs of six mice per group per time point. c. Specific role of type I IFNs on thymic cellularity. IFN-α/βR$^{-/-}$ and IFN-α/βR$^{-/-}$ mice were treated daily i.p. for 3 days with 200 $\mu$g of poly(I:C) or PBS and were sacrificed 3 h after the last injection. Thymi were collected and prepared as single-cell suspensions. Data represent thymic cellularity and absolute counts were obtained by cell numeration. Values of $p$ were calculated from paired Student’s t tests. d. Two hundred micrograms of poly(I:C) treatment leads to a decrease in thymic cellularity, which is maintained over time. Mice were treated i.p. with 200 $\mu$g of poly(I:C) or PBS daily (days 2, 3, 5, and 10) and were sacrificed 3 h after the last injection. Thymi were collected, mechanically disrupted, treated for 10 min with collagenase D, and prepared as single-cell suspensions. Data represent thymic cellularity and absolute counts were obtained by cell numeration. Data are presented as means $\pm$ SD of six mice per group per time point. e. Collapse of DP thymocytes is glucocorticoid independent. Serum corticosterone levels were measured by ELISA 1, 3, 6, and 24 h after i.p. injection of anti-TCRβ (100 $\mu$g), poly(I:C) (200 $\mu$g), and PBS. Values of $p$ were calculated from paired Student’s t tests.
at 24 h posttreatment (7–8 ng/ml; Fig. 1b). IFN-α concentrations were well within the range (30–350 ng/ml) of levels previously reported to be present in the serum of mice during primary viral infections (39, 40). IFN-β production also occurred as early as 3 h posttreatment (2.19 ± 1.03 ng/ml). IFN-β production was also tightly regulated as protein levels returned to low levels at 12 h posttreatment (0.60 ± 0.08 ng/ml) and were undetectable at 24 h posttreatment (Fig. 1b). Similar experiments were preformed in IFN-α/βR−/− mice (24) to establish whether type I IFNs exert an effect on thymic cellularity. These mice show normal thymic development. Thymic cellularity was more severely decreased in wild-type as compared with IFN-α/βR−/− mice (5.7- and 2.3-fold decrease, respectively, p = 0.0047; Fig. 1c). Our results demonstrate that type I IFNs were directly involved in the induction of thymic atrophy, although the role of other cytokines cannot be excluded as suggested by the residual decrease in thymic cellularity observed in IFN-α/βR−/− mice. Kinetic analysis of thymus cellularity and phenotype was performed and extended from 1 to 10 days of poly(I:C) treatment. Of note, a single injection of 200 μg of poly(I:C) followed by the sacrifice of mice 48 h posttreatment did not lead to a thymic atrophy, thus confirming the requirement for repeated injections (data not shown). As expected, thymocyte numbers remained stable in PBS control mice (302–436 × 10⁶ cells). In contrast, poly(I:C)-treated mice showed a 20-fold reduction in thymocyte absolute numbers at day 3 (21.17 × 10⁶ ± 15.09 × 10⁶; Fig. 1d); thymic atrophy was maintained for up to day 10. Interestingly, the decrease in cellularity observed as soon as day 3 in the thymus was not found in mLN, where absolute numbers remained comparable to PBS-treated mice at least until day 10 (see Fig. 5b).

Glucocorticoids severely reduce thymic size and cellularity and can be induced by stress (41, 42). To exclude their potential contribution to poly(I:C)-induced thymic atrophy, mice were injected with 100 μg of either anti-TCRβ (H57-597), 200 μg of poly(I:C), or PBS. Plasma corticosterone concentrations were measured by ELISA 1, 3, 6, and 24 h postinjection. We observed a variable but large rise in plasma corticosterone levels (7.97-fold over background levels) following a 24-h treatment with TCRβ-specific Abs. Levels of poly(I:C)-induced corticosterone concentrations were similar to those induced by injection of PBS (poly(I:C)): 51.3 ng/ml; PBS: 78.0 ng/ml; p = 0.266) and significantly lower than those induced by TCR (TCR: 157.7 ng/ml; p = 0.0132) shown previously to induce thymic atrophy (41); these results indicate that poly(I:C)-induced thymic atrophy occurs independently of the glucocorticoid pathway (Fig. 1e). Altogether, our findings indicate a deleterious impact of poly(I:C) on thymic cellularity; this impact is largely but not exclusively contributed by type I IFNs and to their specific interaction with the IFN-α/β receptor.

**Impairment of early stages of thymopoiesis**

Having shown the negative impact of poly(I:C) treatment on thymic cellularity, we performed experiments to delineate the stages of T cell differentiation targeted by poly(I:C). Poly(I:C) treatment led to the significant accumulation of (1.7-fold) of the earliest colonizing thymocyte precursors, namely, the double negative (DN) I subset as early as 3 days following its administration, leading to a profound impact on early thymic development. Most notably, frequencies of DN2 cells were significantly reduced at day 2 (p = 0.000023), as well as DN3 thymocytes (p = 0.0048; Fig. 2a). Similar results were reproduced (i.e., increase in the frequencies of T cell precursors within DN1) when the latter were further characterized by their expression of c-kit (data not shown) (43). The accumulation of cells in DN1 was also paralleled by a significant reduction in the absolute numbers of all DN subsets (DN1, p = 0.013; DN2, p = 0.002; DN3, p = 0.008; DN4; p = 0.019; Fig. 2b). Taken together, these results suggest a block of thymocyte development at the DN1 to DN2 transition.

Type I IFNs are known for their antiproliferative activity (5), which could in our model also explain the decrease in absolute numbers of early developing thymocytes (Fig. 2b). Mice were injected for 3 days with poly(I:C); BrdU at (1 mg/ml) was added twice at 2-h intervals on the last day of treatment. Thymocytes were harvested and stained with anti-BrdU mAb as described in Materials and Methods. Strikingly, there was a marked decrease of BrdU+ cells in all DN subsets and particularly at the DN3 and DN4 proliferative stages (Fig. 2e). Importantly, frequencies and absolute numbers of DN subsets were not affected upon administration of poly(I:C) to IFN-α/βR−/− mice (Fig. 2d), confirming the contribution of type I IFNs on the impairment of DN thymocyte development. Indeed, percentages of each thymocyte subset remained similar in poly(I:C)-treated mice when compared with control mice injected with PBS in IFN-α/βR−/− mice. Moreover, when treated with poly(I:C), the absolute numbers of DN3 and DN4 cells were significantly higher in IFN-α/βR−/− mice compared with IFN-α/βR−/− mice, demonstrating the direct effect of type I IFNs on the impairment of DN thymocyte development (DN3, p = 0.0017; DN4: p = 0.0155; data not shown). Altogether, our results clearly show that poly(I:C) administration, in large part through the induction of type I IFNs production and their interaction with their specific receptor, leads to demonstrable perturbations of early thymocyte maturation and proliferation.

**Impairment of late stages of thymopoiesis**

Experiments were then performed to identify the impact of poly(I:C) and type I IFNs on the late stages of T cell maturation. DP+ T cells encompass the vast majority of thymocytes (75–85%). Our results (Fig. 3a) showed a very severe reduction in frequencies and absolute numbers of DP thymocytes (day 2: 73.9 ± 2.7%; day 3: 13.0 ± 10.9%, p < 0.000001; Fig. 3a and day 2: 133 × 10⁶ ± 48.9 × 10⁶; day 3: 5.9 × 10⁶ ± 5.2 × 10⁶, p < 0.0001; Fig. 3b), thereby confirming the significant decrease in total thymocyte cell numbers. In parallel, frequencies of CD4 and CD8 SP thymocytes increased as these cells are resistant to type I IFN induced apoptosis (Fig. 3a), in a similar way to peripheral T lymphocytes (44). DP frequencies were significantly less affected in IFN-α/βR−/− mice (57.86 ± 11.19%) than in wild-type mice (37.93 ± 18.36%, p = 0.049), which further confirmed the direct role of the IFNα/β receptor pathway in this perturbation of T cell development (Fig. 3c).

Type I IFNs are also known to be potent inducers of apoptosis (For a review, see Ref. 45). Experiments were conducted to determine whether apoptosis contributed to the thymic atrophy observed in Poly(I:C) treated mice. Results illustrated in Fig. 3d clearly showed a minimal 2-fold increase in Annexing-V+ DP thymocytes, irrespective of the level of expression of the TCR/CD3 coreceptor, a marker of T cell maturation. Indeed levels of TCR are known to increase while the DP subset on T cells undergoes positive and negative selection events (46). Although detected in PBS treated mice (5.18 ± 0.48% and 9.38 ± 2.41% at days 2 and 3, respectively), percentages of Annexing-V+ DP thymocytes were significantly increased following Poly (I:C) treatment (11.75 ± 1.86% (p < 8.10−4) and 23.08 ± 2.83% (p < 4.08.10−4) at days 2 and 3, respectively). DP CD3low, DP CD3int, and DP CD3high subsets all exhibited an increase in the frequency of apoptotic cells at day 2 (Fig. 3d). Lastly, Fig. 3e confirmed the direct impact of type I IFNs on the increased frequencies of apoptotic DP thymocytes as we could not observe any impact to the administration of Poly(I:C) on the percentage of Annexin-V+...
Impairment of early stages of thymopoiesis in poly(I:C)-treated mice.

We then investigated whether the depletion of DP T cells, following treatment with Poly (I:C) led to a biased TCR repertoire. The TCR repertoire is biased following treatment with poly(I:C) and the subsequent induction of Type I IFNs lead to significant perturbations of early and late stages of T cell development.

The TCR repertoire is biased following treatment with poly(I:C)

We then investigated whether the depletion of DP T cells, following treatment with Poly (I:C) led to a biased TCR repertoire. The diversity of the TCR repertoire was evaluated 10 days following the initiation of Poly (I:C) treatment. IFN-α/βR−/− mice were treated daily i.p. for 3 days with 200 μg of poly(I:C) or PBS and were sacrificed 3 h after the last injection. Thymi were collected and prepared as single-cell suspensions, stained for cell surface markers CD3, CD4, CD25, and CD44, and Lin−SAV−CD4−CD8− thymus cells were isolated and evaluated for the level of proliferation, as determined by BrdU uptake by flow cytometry. Representative profiles of six individual mice per group. Number in each quadrant represents the mean of the percentage ± SD for six mice.

 Arrest of the proliferation in DN3-DN4 maturation stages. Mice were treated i.p. with poly(I:C) or PBS daily for 3 days and were sacrificed 3 h after the last injection. For staining with anti-BrdU, animals were injected i.p. twice with 1 mg of BrdU and the thymus was taken 1 h after the last injection. Thymi were collected, mechanically disrupted, treated for 10 min with collagenase D, prepared as single-cell suspensions, and stained for cell surface markers CD3, CD4, CD8, CD25, and CD44. A, Blockade in DN1-DN2 transition. Representative dot plots are shown from gated CD4+/CD8+ thymocytes and numbers in each quadrant represent the mean of percentages obtained for six mice. Absolute counts were obtained by cell numeration and by flow cytometry analysis. Each number represents the mean of the percentage ± SD for six mice. b, Arrest of the proliferation in DN3-DN4 maturation stages. Mice were treated i.p. with poly(I:C) or PBS daily for 3 days and were sacrificed 3 h after the last injection. For staining with anti-BrdU, animals were injected i.p. twice with 1 mg of BrdU and the thymus was taken 1 h after the last injection. Thymi were collected, mechanically disrupted, treated for 10 min with collagenase D, prepared as single-cell suspensions, and stained for cell surface markers CD3, CD4, CD8, CD25, and CD44, and Lin−SAV−CD4−CD8− thymus cells were isolated and evaluated for the level of proliferation, as determined by BrdU uptake by flow cytometry. Representative profiles of six individual mice per group. Number in each quadrant represents the mean of the percentage ± SD for six mice. d, Direct role of type I IFNs on DN maturation stages. IFN-α/βR−/− and IFN-α/βR+/? mice were treated daily i.p. for 3 days with 200 μg of poly(I:C) or PBS and were sacrificed 3 h after the last injection. Thymi were collected and prepared as single-cell suspensions, stained for cell surface markers CD3, CD4, CD8, CD25, and CD44, and assessed by flow cytometry analysis. Representative dot plots for six individual mice are shown from gated CD4+/CD8− thymocytes and numbers in each quadrant represent the mean of percentages obtained for six mice. Values of p were calculated from paired Student’s t tests.
FIGURE 3. Impairment of the late stages of thymopoiesis in poly(I:C)-treated mice. a and b, Mice were treated i.p. with 200 µg of poly(I:C) or PBS 1) daily (days 2, 3, 5, and 10 and were sacrificed 3 h after the last injection) or 2) once and sacrificed 24 or 48 h after injection. Thymi were collected, mechanically disrupted, treated for 10 min with collagenase D, prepared as single-cell suspensions, and stained for cell surface markers CD3, CD4, and CD8 and annexin V. a, Severe and maintained decrease of the frequency of DP thymocytes. Left panel shows the percentages of thymic subsets obtained by flow cytometry analysis: DN (CD4+CD8-), DP (CD4+CD8+), SP CD4+, and SP CD8-. Data represent the mean of six mice per group per time point. Representative dot plots are shown on the right panel. The number in each quadrant represents the mean of the percentage obtained for six mice. b, Absolute number of DP, SP CD4+, and SP CD8- subsets are decreased. Data represent DP subset cellularities after 2, 3, 5, and 10 days of poly(I:C) treatment. Absolute counts were obtained by cell numeration and by flow cytometry analysis. Each number represents the mean of the percentage ± SD for six mice. c, Direct role of type I IFNs on DP decrease in frequency. IFN-α/βR−/− and IFN-α/βR−/− mice were treated daily i.p. for 3 days with 200 µg of poly(I:C) or PBS and were sacrificed 3 h after the last injection. Thymi were collected, mechanically disrupted, treated for 10 min with collagenase D, prepared as single-cell suspensions, and stained for cell surface markers CD3, CD4, and CD8. Left panel, Representative dot plot profiles obtained from flow cytometry analysis of six individual mice per group per time point are shown. The number in each quadrant represents the mean of the percentage for six mice. Right panel, Data represent DP, CD4+, and CD8+ thymocyte subset cellularities. Absolute counts were obtained by cell enumeration and by flow cytometry analysis. Each number represents the mean of the percentage ± SD for six mice. d, Increased apoptosis in DP subsets. Mice were treated i.p. with 200 µg of poly(I:C) or PBS for 3 days and were sacrificed 3 h after the last injection. Thymi were collected, mechanically disrupted, treated 10 min with collagenase D, prepared as single-cell suspensions, and stained for cell surface markers CD3, CD4, and CD8 and annexin V. Results are shown from gated CD4+CD8+ DP thymocyte population. On the basis of levels of CD3 expression, DP CD3low, DP CD3int, and DP CD3high were identified and annexin V levels were obtained on each subset by flow cytometry. Data are presented as means ± SD of six mice per group. e, Direct role of type I IFNs on the increased rate of apoptosis. IFN-α/βR+/+ and IFN-α/βR−/− mice were treated daily i.p. for 3 days with 200 µg of poly(I:C) or PBS and were sacrificed 3 h after the last injection. Thymi were collected, mechanically disrupted, treated for 10 min with collagenase D, prepared as single-cell suspensions, and stained for cell surface markers CD3, CD4, and CD8 and annexin V. Data are presented as means ± SD of six mice per group.
The TCR repertoire is biased following treatment with poly(I:C). a–c, Mice were treated daily i.p. with 200 μg of poly(I:C) or PBS for 10 days and sacrificed 3 h after the last injection (“atrophied thymus”). Thymi were collected, mechanically disrupted, treated for 10 min with collagenase D, prepared as single-cell suspensions, and stained for the cell surface markers CD3, CD4, CD8, and CD24. a, Gating strategy of cell sorting of CD3\(^+\)CD4\(^+\)CD24\(^{int}\) and CD3\(^+\)CD8\(^+\)CD24\(^{int}\) thymocytes. CD24\(^{int}\) cells have been reported to be both differentiated and nonrecirculating from the periphery (47). We excluded CD24\(^+\) cells from our flow cytometry analysis since the expression of this marker is lost with maturation and absent in peripheral T cells. b and c, SP CD4\(^+\) and CD8\(^+\) T cell repertoire of “long-term atrophied thymus.” Twenty V\(\beta\) immunoscopes (V\(\beta\)-1, 2, -3, -4, -5.1, -5.2, -6, -7, -8.1, -8.2, -8.3, -9, -10, -11, -12, -13, -14, -15, -16, -18, and -20) were generated by RT-PCR from cells sorted based on the cell surface phenotype from three individual mice per group. In Fig. 4b are shown 6 of 20 V\(\beta\)s. PCR were repeated at least twice to confirm that the absence of a positive signal was not due to PCR artifacts. V\(\beta\)-C\(\beta\) spectratype profiles are represented by a black spot when biased. Several levels of CDR3 spectratype alterations were found: some V\(\beta\) genes were not biased (zero of three), others were biased in only one of three mice, two of three mice, or three of three mice. Analysis of biases for a given V\(\beta\) gene implied that the three PBS control mice exhibited superimposed V\(\beta\)-C\(\beta\) profiles. When this was not the case, analysis of the subsequent V\(\beta\) was considered as undefined (U). d, Reduction of TdT mRNA relative expression in DP\(^+\) thymocytes after 3 days of poly(I:C) treatment. Mice were treated daily i.p. with 200 μg of poly(I:C) or PBS for 3 days and were sacrificed 3 h after the last injection. Thymi were collected, mechanically disrupted, treated for 10 min with collagenase D, prepared as single-cell suspensions, and stained for the cell surface markers CD3, CD4, CD8, and DP\(^+\). Cells were FACS purified using cell surface markers CD3, CD4, and CD8. TdT, Rag2, and helios expression were measured by semiquantitative PCR. Signal intensity of TdT, Rag2, and helios were reported to signal intensity of β-actin for each mouse. Quantifications were performed with the ImageQuant software. At least five mice per group per time point were analyzed for this experiment.
FIGURE 5. Arrest of thymus function rapidly affects thymic output. a, Increased retention of SP T cells within the thymus and mLNs after poly(I:C) treatment. Mice were treated i.p. with 200 μg of poly(I:C) or PBS daily for 3 days and were sacrificed 3 h after the last injection. Upper panel, Thymi were collected, mechanically disrupted, treated for 10 min with collagenase D, prepared as single-cell suspensions, stained for cell surface markers CD3, CD4, CD8, and CD69, and assessed by flow cytometry. Shown is the MFI of CD69 in DP, SP CD4+, and SP CD8 thymocyte subsets. Lower panel, mLNs were collected, mechanically disrupted, treated for 10 min with collagenase D, prepared as single-cell suspensions, stained for cell surface markers and CD3, CD4, CD8, CD44, and CD62L, and assessed by flow cytometry. Shown is the MFI of naive CD4+, memory CD4+, naive CD8+, and memory CD8+.

Representative histograms of six individual mice per group per time point are shown. The numbers in each quadrant represent the average of the MFI values for six mice. b and c. Mice were treated daily i.p. with 200 μg of poly(I:C) for 2, 3, 5, or 10 days and were sacrificed 3 h after the last injection. mLNs were collected, mechanically disrupted, treated for 10 min with collagenase D, prepared as single-cell suspensions, and stained for cell surface markers CD3, CD4, CD8, CD44, and CD62L. b, Decrease in absolute numbers of the T naive subset in mLNs. Data represent total (upper panel), naive CD8+ (middle panel), and naive CD4+ subset (lower panel) cellularity after 2, 3, 5, and 10 days of poly(I:C) treatment. Absolute counts were obtained by cell numeration and by flow cytometry analysis. Each number represents the mean of the percentage ± SD for six mice per time point. c, Decrease of naive T cells is not attributed to increased T cell death in periphery. Mice were treated daily i.p. with 200 μg of poly(I:C) for 3 or 5 days and were sacrificed 3 h after the last injection. mLNs were collected, mechanically disrupted, treated for 10 min with collagenase D, prepared as single-cell suspensions,
(Vβ2, Vβ7 and Vβ15). Of note, most biased Vβs were characterized by the absence of longer CDR3 peaks (Fig. 4b, data not shown). Representative examples for Vβ2, Vβ7 and Vβ8.3 are illustrated in Fig. 4b. Lastly, as summarized in Fig. 4c, CD4+ and CD8+ T cell repertoires were biased in ~55–65% of the 21 Vβ analyzed genes.

We next explored whether the observed absence of long CDR3 Vβ chains could have resulted in part from the reduction of VDJ recombination activity. The expression of TdT (mediating N nucleotide addition at the V-D and D-J junction) and Rag2 (expressed specifically in cells undergoing the recombination of TCR genes) mRNA was thus quantified by semiquantitative RT-PCR in FACS sorted DP thymocytes obtained from PBS and Poly (I:C) treated mice after 3 days of treatment. Strikingly, Poly (I:C) treatment led to a significant reduction of TdT (p = 0.044) and Rag2 (p = 0.022) mRNA whereas the gene control Helios was similar in both groups (p = 0.42) (Fig. 4d). These results were confirmed in sorted DN3-DN4 thymocytes (data not shown) which are the primary stage of T cell differentiation where TCR gene rearrangement occurs. Altogether, these results showed that the inhibition of VDJ recombination associated gene expression leads to the restriction of the T cell repertoire in exiting thymocytes from poly(I:C) treated mice, characterized mostly by a shift toward shorter CDR3 lengths.

**Arrest of T cell development rapidly affects thymic output**

We then aimed at verifying the impact of the perturbations in thymopoiesis on thymic output to the periphery. Type I IFNs are strong inducers of the cell surface activation marker CD69; the latter inhibits thymocyte egress by forming a complex with the chemokine receptor S1P1 thereby negatively regulating its cell surface expression and function (48) (49, 50). Experiments were then performed to define whether treatment with poly(I:C) led to a decrease in the absolute number of naive T cells in mLN indicated that the bulk decrease in exit thymocytes to the peripheral compartment. Indeed, both sjTRECs (sj58 and sj61) and DJβ1 (1.1–1.6) and DJβ2 (2.1–2.7) TRECs number per 107 cells were all reduced in mLN of poly(I:C)-treated mice (Fig. 5d). Altogether, these results provide strong evidence for a substantial diminution of thymic output induced by poly(I:C) treatment.

An independent approach to evaluate thymic output consists in measuring the production rate of recent thymic emigrants (RTE) in Rag-2p-GFP mice (51). These mice are transgenic for the GFP driven by the Rag2 promoter, allowing the detection of GFPbright RTE in the periphery. Poly(I:C) treatment was applied for 5 or 10 days to these mice. After day 10 of poly(I:C) administration (PBS, 34.50 ± 10.09 × 10^6; poly(I:C), 23.00 ± 4.58 × 10^6; p = 0.131), the absolute numbers of GFP+ T cells in mLN were markedly reduced in both CD4+ (PBS, 2.08 ± 0.32 × 10^6; poly(I:C), 1.08 ± 0.47 × 10^6; p = 0.019) and CD8+ subsets (PBS, 0.48 ± 0.07 × 10^6; poly(I:C), 0.17 ± 0.09 × 10^6; p = 0.003; Fig. 5e). Concomitantly, frequencies of apoptotic cells remained unchanged in CD4+GFP+ and CD8+GFP+ cells.

To exclude the possibility that the decrease in the numbers of naive T cells in mLN might be due to a peripheral redistribution of cells rather than the consequence of the arrest of thymic output, we repeated poly(I:C) treatment for 5 days and measured the cell surface expression level of CD69 (Fig. 5f). CD69 expression was significantly increased after poly(I:C) treatment in all tested T cell subsets, including naive CD4+ and CD8+ T cells (naive CD4+, p = 0.0002; memory CD4+, p < 0.0001; naive CD8+, p < 0.0001; memory CD8+, p < 0.0001). These results clearly indicated that after a peak of production of type I IFNs, peripheral T cells were also preferentially sequestered within the mLN. Consequently, the severe decrease of naive T cell absolute numbers observed after 10 days of treatment could not be attributed to an escape of this subset from mLN but rather to the reduction of thymic output. It is however difficult to exclude the contribution of low levels of T cell death to the observed decrease of naive T cells in periphery. Such levels would be below those detected by annexin V. Altogether, our results demonstrate that the severe decrease of thymopoiesis is rapidly followed by a reduction in the numbers of the RTE/naive pool in the periphery.
Maintenance of a complete thymopoiesis potential of poly(I:C)-treated thymus

We then investigated whether thymic function was irreversibly altered in poly(I:C)-treated mice. C57BL/6 mice were treated with poly(I:C) for 3 days and sacrificed 0, 7, 14, 21, or 28 days after the last injection. Thymi and mLNs were collected, mechanically disrupted, treated for 10 min with collagenase D, and prepared as single-cell suspensions and absolute counts were obtained. Then, thymi were stained for cell surface markers CD25, CD44, CD3, CD4, CD8, and annexin V. Return to normal levels of thymus and mLN cellularity. Absolute counts were obtained by cell enumeration. Data are presented as means ± SD of six mice per group per time point. Kinetics return to normal frequencies of thymocyte subsets after interruption of poly(I:C) treatment. Upper panel, Representative dot plots obtained by flow cytometry analysis of CD4^+CD8^- DP, SP CD4^+, and SP CD8^- thymocyte subset frequencies are shown and the number in each quadrant represents the mean of the percentage for six mice. Lower panel, Representative dot plots obtained by flow cytometry analysis of DN1, DN2, DN3, and DN4 frequencies are shown from gated CD4^+CD8^- DN subsets and the number in each quadrant represents the mean of the percentage for six mice.

Maintenance of a complete thymopoiesis potential of poly(I:C)-treated thymus

We then investigated whether thymic function was irreversibly altered in poly(I:C)-treated mice. C57BL/6 mice were treated with poly(I:C) for 3 days and sacrificed 0, 7, 14, 21, or 28 days after cessation of treatment. When T cell counts were examined as a function of time after discontinuation of poly(I:C) treatment, we could not show any recovery in T cell numbers observed after 7 days (8.23 ± 3.1 × 10^6 cells). However, by 14 days after cessation of treatment, mice showed a complete recovery (245.67 ± 39.55 × 10^6 cells; Fig. 6a). Interestingly, assessment of thymic cellularity following the reconstitution phase showed an increase in thymocyte numbers as compared with PBS-treated mice at day 21 (PBS, 311 ± 65 × 10^6; poly(I:C), 410 ± 73 × 10^6; p = 0.032) and day 28 (PBS, 248 ± 75 × 10^6; poly(I:C), 383 ± 53 × 10^6; p = 0.014).

We then examined the kinetics and quantitative and qualitative...
features of thymic reconstitution. Our results show that by day 21, most of thymic integrity at the phenotypic level is restored. Indeed, DN, DP, and CD4 thymocyte subset distribution was back to values comparable to those observed in PBS-treated mice (DN: PBS = 2.93%; poly(I:C) = 2.48%, p = 0.132; DP: PBS = 73.77%, poly(I:C) = 72.68%, p = 0.739; SP CD4+; PBS = 5.08%, poly(I:C) = 4.90%; p = 0.621; and SP CD8+; PBS = 1.72%, poly(I:C) = 1.25%, p < 0.001; Fig. 6b). Altogether, these data clearly provided evidence for a preserved thymopoiesis potential in mice that have been treated with poly(I:C) following cessation of this treatment.

We next investigated the impact of restoration of thymic activity on thymic output. As a consequence of thymic recovery, progressive mLN replenishment was observed over time (Fig. 6a). It corresponded to a progressive increase in the numbers of CD44+CD62L+CD4+ and CD44+CD62L+CD8+ naïve T cells, although the CD8+ subset remained still significantly reduced 28 days after stopping poly(I:C) treatment (naive CD4+ (day 14): PBS = 0.81 × 10^6, poly(I:C) = 0.11 × 10^6, p < 0.001; day 28: PBS = 1.03 × 10^6, poly(I:C) = 0.68 × 10^6, p = 0.052 and naive CD8+ (day 14): PBS = 2.71 × 10^6, poly(I:C) = 0.82 × 10^6, p < 0.001; day 28: PBS = 2.56 × 10^6, poly(I:C) = 1.57 × 10^6, p = 0.007). Moreover, the defect observed in sjTREC levels in mLN was completely abolished 21 days after cessation of poly(I:C) administration (Fig. 6c). In conclusion, we have shown the restoration of thymic integrity and function, as a consequence of stopping the administration of poly(I:C).

We then asked whether this return of thymic activity was accompanied by the regeneration of an unbiased TCR repertoire. CDR3 spectratypes were performed at this time point (as described in Fig. 4b). The number of biased Vβ subtypes drastically decreased compared with the 10-day atrophied thymus, even if few biased Vβ CDR3 repertoire still persisted in the CD4+ subset (data not shown). Interestingly, none of the observed biases were characterized by the absence of long CDR3 peaks in mice treated with poly(I:C), which was indicative at least of a complete return to normal TCR diversity (76.19% Vβ gene did not show detectable bias; Fig. 6d and data not shown). Altogether, our results clearly demonstrate that impairment of thymic function can be almost completely reversed when poly(I:C) treatment is arrested.

Discussion

In the present report, we have aimed at characterizing the impact of a virally encoded TLR3/RIG-I ligand on the homeostasis of thymic function. We have provided evidence for the early, direct, and detrimental impact of poly(I:C) treatment on thymopoiesis. This impact could be attributed to several mechanisms which act at both the DN and DP stages. Ultimately, the induced thymic atrophy led to a severe decrease of thymic output (i.e., frequency of RTE) and also to alterations of qualitative properties of the RTE as shown from the decrease in the diversity of the TCR repertoire. The fact that these alterations in T cell development and thymic output were mostly abolished in IFN-α/βR−/− mice provides the evidence of the contribution of type I IFNs in qualitatively and quantitatively modulating the TCR repertoire of de novo-produced T cells.

Poly(I:C) treatment perturbs early and late stages of thymic development

We have previously shown that poly(I:C) treatment leads to three successive and organized waves: early IFNs (IFN-β and α4), late IFNs (IFN-α1, α2, and α5), and “secondary late IFNs” (IFN-α6T and α8/6) (T. Démoïlus, N. Kettata, A. Abdallah, and R.-P. Sékaly, manuscript submitted). This prompted us to use repeated injections of poly(I:C) to induce this protein cascade instead of administering a unique IFN-α subtype as this would probably not entirely mimic a virally induced type I IFN response, also characterized by several waves of IFN-α subtypes. The negative impact of type I IFNs on thymic development was already observed on the first cells colonizing the thymus (17); furthermore, we clearly demonstrated that this is mostly a consequence of both a blockade of the DN1-DN2 transition and a complete arrest of DN3-DN4 proliferation (Fig. 2). The decrease in DN proliferation could result from the killing of thymic epithelial cells (TECs) by type I IFNs (52). TECs play an important role on early stages of thymic development as they have been shown to play a critical role in regulating the proliferation and survival of the most immature thymocytes through the release of different mediators including, IL-7 and keratinocyte growth factor (53–55). Moreover, it is possible that the IL-7R could be down-regulated in poly(I:C)-treated mice. Indeed, thymocytes from murine FTOCs treated with poly(I:C) show significantly decreased levels of IL-7R subsequent to the administration of poly(I:C) and specifically in DN1/DN2 cellular subsets (19). From these results, it clearly appears that the induced atrophy of the thymus results from TCR-independent effects of poly(I:C) on the early stages of thymopoiesis.

Poly(I:C) led to a severe reduction of the number of DP T cells. It is likely that the above-reported accumulation in DN1 thymocytes and the decreased proliferation and differentiation of DN3-DN4 thymocytes could partly explain the loss of DP cells as described in Fig. 3. It is also likely that the increased death of DP thymocytes may result from the direct induction by type I IFNs of the prosapoptotic proteins. Bax and Bak, which are direct transcriptional targets of type I IFNs (56), and have also been implicated in apoptosis leading to thymocyte negative selection (57).

The direct impact of type I IFNs on the inhibition of T cell development was confirmed by treating FTOCs with individual IFN-α subtypes (i.e., DN blockade, arrest of extensive DN proliferation, and increased number of apoptotic cells) (19). However, in our poly(I:C) model, thymic atrophy was significantly diminished although not completely abolished in IFN-αR−/− mice, suggesting the involvement of other factors. Interestingly, we have observed a sharp increase in serum of TNF-α using cytometric bead array 3 days after initiating poly(I:C) treatment (data not shown). The fact that TNF-α was shown to be produced in response to poly(I:C) (58–60) and was reported to impair T cell development by inducing apoptosis on DN3-DN4 (61) and SP CD4+ and CD8+ (62) identifies it as another potential contributing factor to thymic development in mice treated with poly(I:C).

Impact of poly(I:C) on the diversity of the de novo-produced T cell repertoire

We next determined whether the collapse of DP cells number could drive the generation of an aberrant T cell repertoire. It is known that DP thymocytes migrate for 3–5 days in the cortex to interact with TECs and undergo positive selection. We thus investigated the TCR repertoire at day 10 of treatment, a time point which, as we have shown, impacts on thymic selection processes. We provided evidence that 55–60% of Vβ rearrangements were biased in terms of CDR3 length repartition. In most cases, these biases were characterized by the absence of long CDR3 size length rearrangements. This absence of long CDR3 Vβ chains resulted from the reduction of VDJ recombination activity and specifically of the N nucleotide addition mediated by the TdT (Fig. 4d). In agreement with our results, Cabaniols et al. (63) have shown that TdT−/− mice display a striking shift toward shorter CDR3 Vβ chains lengths, leading to a 10-fold reduction of the diversity of the
Vß repertoire. Interestingly, a previous report showed the inhibition of TdT activity in human T lymphoblastoid cell lines when treated with IFN-α (64). Finally, the reversibility of the alteration of the T cell repertoire that we observed 14 days after cessation of treatment confirms a previous study where IFN-α was suggested to delay rather than prevent TCRß rearrangements (20). Collectively, our data demonstrate overall changes in the primary structure of CDR3 TCR-ß chains that could impact on the recognition of foreign peptides by TCR. Indeed, T cells from TdTGFP mice show decreased reactivity to foreign Ag when compared with the TdTGFP counterparts (65).

Consequences of the level of thymopoiesis on thymic output

The administration of poly(I:C) led to a marked reduction of thymopoiesis, which resulted in a significant decrease of de novo thymic output and consequently numbers of naive T cells were decreased. Quantification of RTE requires highly sensitive tools as thymic output and consequently numbers of naive T cells were decreased reactivity to foreign Ag when compared with the TdTGFP counterparts (65).

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References


