

The Journal of Immunology

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This information is current as
of June 9, 2010

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J. Immunol. published online May 19, 2010;
doi:10.4049/jimmunol.0903109

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Control of T Cell Reactivation by Regulatory Qa-1–Restricted CD8⁺ T Cells

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Administration of attenuated pathogenic T cell clones, a procedure known as T cell vaccination, induces CD8⁺ T cells specific for peptides derived from the V β -chain of the TCR presented by the MHC class Ib molecule, Qa-1 expressed on the vaccine cells. These regulatory CD8⁺ T cells have the capacity to control the activation of endogenous T cells expressing the same TCR V β -chain as the vaccinating cells. We hypothesized that vaccination with NKT cells could also induce Qa-1–restricted CD8⁺ T cells that would control NKT cell activation. We tested this hypothesis in a murine model of Con A-induced hepatitis that is induced by NKT cells. Vaccination with NKT cells effectively induced protective Qa-1–restricted CD8⁺ T cells that prevented hepatitis. Surprisingly, upon vaccination with T cells expressing V β -chains irrelevant to NKT cells, we discovered that the specificity of vaccine-induced Qa-1–restricted CD8⁺ T cells was not limited to the V β -chain of the vaccinating cells. We further show that these regulatory Qa-1–restricted CD8⁺ T cells arise spontaneously upon polyclonal activation of T cells in the absence of deliberate T cell vaccination. These experiments provide new insight into a CD8⁺ T cell compartment that regulates the immediate reactivation of conventional T cells and NKT cells. *The Journal of Immunology*, 2010, 184: 000–000.

To keep T cell autoreactivity in check, the immune system has evolved several mechanisms, some of which involve networks of regulatory T cells that keep peripheral T cells self-ignorant (1). Nonclassical MHC class Ib molecules, such as Qa-1 in mice and HLA-E in humans, are increasingly highlighted as molecules belonging to such a regulatory network. Surface Qa-1 is loaded with a dominant peptide derived from the MHC class I leader sequence known as the Qdm peptide (2). Interaction between Qa-1–Qdm complexes with CD94–NKG2A heterodimeric killer inhibitory complexes on NK cells and CD8⁺ T cells (3) inhibits killer cell activity (4). Qa-1–Qdm complexes expressed by cells secondary to the release of leader sequences (Qdm) from newly formed MHC class I molecules can thereby be considered as a signal to CD8⁺ T cells and NK cells that the classical MHC I loading compartment is functioning properly.

Upon activation of T cells, the Qdm peptide is rapidly replaced by other peptide sequences derived from a range of proteins, such

as preproinsulin (5), heat shock protein 60 (6), and some bacterial proteins (7). Qa-1 molecules can also be loaded with V β peptides derived from the TCR of activated T cells (8, 9). Remarkably, such activated Qa-1–expressing T cells (referred to hereafter as vaccinating cells) are used as immunointervention tools, because they are capable of inducing a regulatory T cell response, in a procedure termed T cell vaccination (TCV) (10). TCV has been shown to induce Qa-1–restricted CD8⁺ T cells that control the expansion of endogenous CD4⁺ T cells expressing Qa-1–V β peptide complexes similar to those expressed by vaccinating cells (8, 11–13).

Current studies using TCV are focused on the induction of regulatory T cells specific for the V β clonality of the vaccinating T cells. However, for a given disease, the V β clonality of the pathogenic T cells is specific to each individual. To be applicable, TCV should be tailored to each individual. Alternatively, to broaden the scope of TCV therapy for certain diseases, it might be possible to target pathogenic T cells that belong to restricted V β families. In this study, we tested the possibility of targeting one such restricted population: the NKT cells.

Invariant NKT cells (referred to hereafter as NKT cells) are unconventional T cells that express a limited repertoire of V β -chains in their TCR. Mouse NKT cell TCR contains the V β 2, 7, 8.2, or 10 chains (14). Remarkably, in humans V β 11 is the only known V β -chain in NKT cell TCRs (14). NKT cells are pathogenic in a number of diseases (14, 15). Targeting these cells by the TCV approach might therefore serve as a potential therapeutic tool for certain immunologic disorders.

We used the mouse model of NKT cell-mediated Con A-induced hepatitis to demonstrate that NKTCV induces regulatory Qa-1–restricted CD8⁺ T cells. The protection conferred did not depend on the V β clonality of the vaccinating cells used and demonstrated the existence of Qa-1–restricted CD8⁺ T cells capable of controlling the activation of T cells. We further demonstrate that these regulatory CD8⁺ T cells are normally induced in primary T cell responses and can control a secondary activation of the

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Received for publication September 21, 2009. Accepted for publication April 1, 2010.

This work was supported by the Institut National de la Santé et de la Recherche Médicale and the Fondation de France.

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Abbreviations used in this paper: Alat, alanine aminotransferase; Asat, aspartate aminotransferase; Ctrl, control; ns, nonsignificant; KO, knockout; PLSD, protected least significant difference; TCV, T cell vaccination.

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T cells by the same stimulus within a short time lapse. These results unravel a regulatory mechanism used by the adaptive immune system to tightly control immediate reactivation of T cells.

Materials and Methods

Mice

Six-week-old male C57BL/6 (H-2^b, Qa-1^b) mice and the first generation of a cross between C57BL/6 (H-2^b, Qa-1^b) and CBA/J (H-2^k, Qa-1^b) mice (B6CBA/F1, H2^{b/k}, Qa-1^{b/b}) were purchased from Janvier Laboratories (Le Genest-St-Ile, France). 5CC7 TCR transgenic RAG knockout Vβ3 TCR transgenic mice (H-2^k, Qa-1^b) were a gift from Dr. Corinne Tanchot (INSERM Hôpital Necker, Paris, France). Generation of Qa-1-deficient mice has been previously described (16). During the course of the experiments, all mice were maintained in pathogen-free conditions and all experiments were approved by the institutional ethical committee.

Cell lines

CD4⁺ T cell hybridomas 96-6 (Vβ6⁺, H-2^{b/k}, Qa-1^b) and D7 (Vβ7⁺, H-2^{b/k}, Qa-1^b) were produced by fusion of primary T cells with the BW5147 thymoma cell line. The NKT cell hybridomas, NKT1-2 (Vβ8⁺, H-2^{b/k}, Qa-1^b) and NKT1-4 (Vβ10⁺, H-2^{b/k}, Qa-1^b) were a kind gift from Dr. Mitchell Kronenberg (La Jolla Institute of Allergy and Immunology, San Diego, CA) and have been described previously (17). The melanoma cell line B16.F1 (H-2^b, Qa-1^b) has been described previously (18).

T cell vaccination

T cell hybridomas, BW5147 cells, and primary CD4⁺ T cells from the spleen were stimulated *in vitro* using 5 μg/ml of Con A (C2010; Sigma Aldrich, Lyon, France) for 40 h in RPMI 1640 (Life Technologies, Rockville, MD) medium containing 10% FCS at 37°C. The B16.F1 cells were activated with IFN-γ (200 IU/ml) for 40 h in DMEM (Life Technologies) medium containing 10% FCS at 37°C. All cells were washed thoroughly with RPMI 1640 or DMEM without FCS. T cell hybridomas and primary T cells were irradiated at 3000 rad, and BW5147 and B16.F1 cells were irradiated at 20,000 rad. Preliminary experiments confirmed that cell proliferation was blunted at these doses (data not shown). Two and a half million activated and irradiated cells resuspended in RPMI 1640 without FCS were injected retro-orbitally into mice. Control mice were injected with 200 μl RPMI 1640 alone.

Isolation of lymphoid cells and flow cytometry

Single cell suspensions of the spleen, lymph nodes, and liver were prepared by meshing the organs through a 100-μm nylon filter. Liver mononuclear cells were obtained by performing a Percoll 37.5% (P1644; Sigma Aldrich) gradient centrifugation of the cell suspension. RBCs were lysed in the liver and spleen cell suspensions using ACK lysis buffer.

Abs and reagents

Monoclonal Abs specific for CD4 (clone RM4-5) and CD8 (clone 53-6.7) at a concentration of 2 μg/ml were used in this study. NKT cells were identified using α-galactosylceramide-loaded CD1d tetramers (1/200 dilution) incubated for 1 h at 4°C. Flow cytometric acquisition was performed using a BD LSRII (BD Biosciences, Le Pont de Claix, France) and the data were analyzed using BD Diva software (BD Biosciences).

Adoptive transfer of purified CD8⁺ T cells

Purification of CD8⁺ T cells from splenocytes was performed using the BD CD8⁺ T cell enrichment kit (BD Biosciences, San Jose, CA; 558471). CD8⁺ T cell purity was analyzed by flow cytometry to be ≥90%. Before transfer, CD8⁺ T cells were labeled with CFSE (Molecular Probes, Eugene, OR; C1157) at a concentration of 25 μM, and 6 to 8 million cells were injected retro-orbitally into naive 6-wk-old male C57BL/6J or B6CBA/F1 mice.

Induction of hepatitis

Hepatitis was induced by injecting Con A (C2010; Sigma Aldrich) in PBS 1×, retro-orbitally at a dose of 10 mg/kg of body weight. Blood was collected from the periorbital sinus or from the left ventricle in sodium heparin anticoagulant (Venoject, Laboratoires Terumo, Guyancourt, France) at 5, 8, 16, or 24 h after Con A injection.

Plasma enzyme analysis

Plasma was separated by centrifugation. The activity of hepatic enzymes alanine aminotransferase and aspartate aminotransferase in the plasma was

measured using an Olympus AU400 bioanalyzer (Beckman Coulter, Villepinte, France).

Statistics

Data are presented as mean ± SEM. Differences between the means were evaluated using the nonparametric Mann-Whitney *U* test or ANOVA Fishers protected least significant difference (PLSD) test where applicable and were considered significant at *p* < 0.05.

Results

NKT cell vaccination protects mice against Con A-induced hepatitis

To test whether we can target NKT cells using the TCV approach, we adopted the experimental model of murine hepatitis induced upon *i.v.* injection of ConA. The subsequent NKT cell-dependent Fas-FasL interaction-mediated destruction of hepatocytes leads to fulminant hepatitis (19–21). We vaccinated mice with activated and irradiated NKT cell hybridomas expressing two different Vβ-chains, Vβ8 (NKT1-2 cells) or Vβ10 (NKT1-4 cells). We found that the use of T cell hybridomas facilitates the TCV procedure that requires the injection of a large number (2.5 million) of monoclonal vaccinating cells. Given their mixed H-2^{b/k} haplotypes, hybridomas require to be administered in H-2^{b/k} hosts such as the B6CBA/F1 (C57BL6/J × CBA/J) mice used in this study. After 3 wk, hepatitis was induced in NKT cell-vaccinated mice and the activity of hepatic enzymes alanine aminotransferase and aspartate aminotransferase in the plasma were used to score the disease. As expected, the nonvaccinated control mice developed acute fulminant hepatitis (Fig. 1). On the contrary, all mice vaccinated with either Vβ10⁺ NKT or Vβ8⁺ NKT cell hybridomas were significantly protected from hepatitis (Fig. 1), demonstrating the possibility of controlling an NKT cell-mediated disorder using a procedure that we named NKTCV.

Protection conferred by NKTCV is not Vβ-chain-restricted

To test whether NKTCV-induced protection was specific for the Vβ-chain of NKT cells, we vaccinated mice with primary Vβ3⁺ CD4⁺ T cells from TCR transgenic mice or Vβ6⁺ or Vβ7⁺ conventional CD4⁺ T cell hybridomas (established from conventional primary T cell clones fused with the same cell partner used for the NKT cell hybridomas, the T cell lymphoma BW5147). It is to be noted that invariant NKT cells do not express Vβ3 or Vβ6 chains

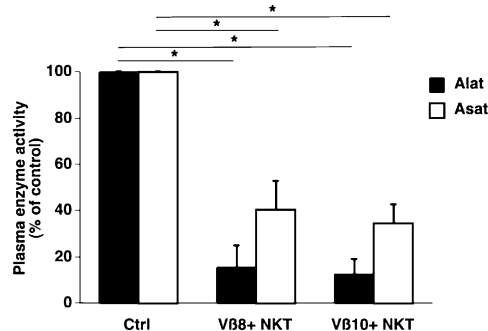


FIGURE 1. Vaccination with NKT cells protects mice from hepatitis. B6CBA/F1 mice were injected *i.v.* with PBS (Ctrl, *n* = 6) or Con A-activated and irradiated Vβ8⁺ (Vβ8⁺ NKT, *n* = 7) or Vβ10⁺ (Vβ10⁺ NKT, *n* = 6) NKT cell hybridomas. Three weeks later, hepatitis was induced by *i.v.* injection of Con A (10 mg/kg). The figure depicts the percentage of plasma alanine aminotransferase (Alat, black bars) and aspartate aminotransferase (Asat, white bars) activity measured 8 h after Con A injection, as compared to the controls. *Statistical significance between interconnected groups evaluated with *p* < 0.05 calculated using the nonparametric Mann-Whitney *U* test (mean ± SEM). Ctrl, control.

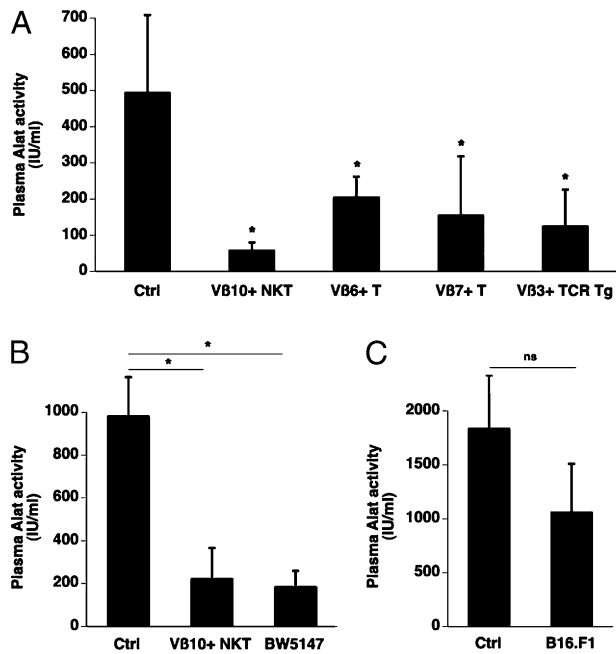


FIGURE 2. Protection from hepatitis is conferred by T cells independent of the expression of particular TCR V β -chains. **A**, B6CBA/F1 mice were vaccinated with PBS (Ctrl, $n = 5$) or Con A-activated and irradiated V β 10⁺ NKT cell hybridomas (NKT V β 10⁺, $n = 4$), V β 6⁺ (V β 6⁺ T, $n = 4$) or V β 7⁺ (V β 7⁺ T, $n = 4$) T cell hybridomas, or primary V β 3⁺ CD4⁺ T cells from TCR transgenic mice (V β 3⁺ Tg, $n = 3$) followed by the induction of hepatitis 3 wk later. The figure depicts the plasma AlAt activity in IU/ml measured 8 h after Con A injection. **B**, Plasma AlAt enzyme activity in mice vaccinated with PBS or activated and irradiated V β 10⁺ NKT cells ($n = 5$) or BW5147 cells ($n = 5$) measured 8 h after Con A (10 mg/kg) injection. **C**, Plasma AlAt enzyme activity in mice vaccinated with PBS (Ctrl, $n = 5$) or activated and irradiated B16.F1 cells (B16.F1, $n = 5$) measured 8 h after ConA (10 mg/kg) injection. Concordant results were obtained with Asat levels (data not shown). *Statistical significance of each group compared with the Ctrl group with $p < 0.05$, calculated using the nonparametric Mann-Whitney U test (mean \pm SEM).

in their TCR (14). As before, the disease was induced by an i.v. injection of ConA, 3 wk after vaccination. Surprisingly, we found that all vaccinated mice, irrespective of the V β -chain expressed by the vaccinating cells, were protected against hepatitis (Fig. 2). This finding indicates that in this model, protective responses induced by both NKTCV and TCV does not rely on the type of V β -chain expressed by the vaccinating cells. Concordantly, the fusion partner BW5147 T cell lymphoma that lacks the expression of a TCR was able to confer equal protection against Con A-induced hepatitis (Fig. 2B). The equally potent protection conferred by primary T cells (V β 3⁺) ruled out the possibility that NKTCV- or TCV-induced protection relies on features specific to hybridomas and indicates that T cell hybridomas are able to induce efficient vaccination effects. Furthermore, activated melanoma cells, B16.F1, failed to confer protection (Fig. 2C), indicating that the target epitopes that induced protection were specific for the T cells.

Protection conferred by NKTCV is performed by CD8⁺ T cells

Whereas our experiments indicated that NKTCV induced efficient protection independently of the V β -chain expressed by the vaccinating cells, we aimed at determining whether it was dependent on CD8⁺ T cells, akin to TCV. To investigate, we adoptively transferred CD8⁺ T cells from either NKT or T cell-vaccinated mice to naive mice. Mice that received CD8⁺ T cells from control non-vaccinated mice developed fulminant hepatitis. Interestingly, the

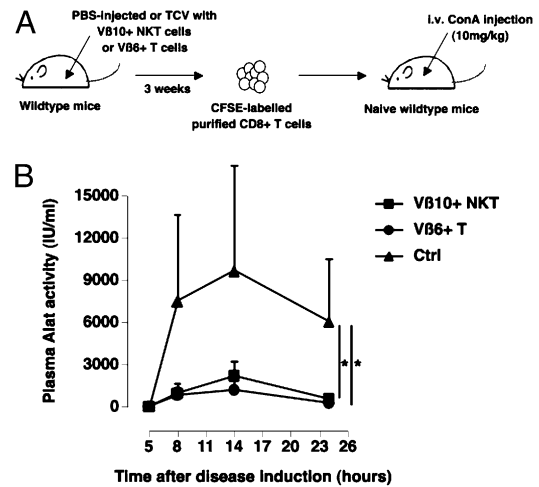


FIGURE 3. CD8⁺ T cells induced upon NKTCV and TCV confer protection against hepatitis. **A**, Representation of the experimental design; 7.8 million CD8⁺ T cells purified from the spleen of nonvaccinated B6CBA/F1 mice (Ctrl, triangles, $n = 5$) or mice vaccinated with V β 10⁺ NKT (V β 10⁺ NKT, squares, $n = 5$) or V β 6⁺ T (V β 6⁺ T, circles, $n = 5$) cell hybridomas were adoptively transferred to naive mice. Hepatitis (ConA 10 mg/kg) was induced in the recipient mice 16 h later. **B**, Plasma AlAt activity measured 5, 8, 14, and 24 h after Con A injection. *Statistical significance with $p < 0.05$, calculated using the ANOVA Fishers PLSD test (mean \pm SEM).

recipients of CD8⁺ T cells from both groups of vaccinated mice were protected from Con A-induced hepatitis (Fig. 3). Protection conferred against NKT cell-mediated pathology by CD8⁺ T cells from mice vaccinated with V β 6⁺ T cell hybridomas confirms that these cells recognize V β -independent targets.

Previous studies have shown that the population of liver NKT cells undergoes massive activation-induced cell death upon Con A injection (19). Accordingly, upon hepatitis induction in nonvaccinated mice, we observed a reduction of the number of NKT cells in the liver detected by α -galactosylceramide (α -galcer)-loaded CD1d tetramers ($1.1 \pm 0.3\%$ of NKT cells among total liver mononuclear cells, compared with $6.2 \pm 0.2\%$ in normal untreated B6CBA/F1 mice). In comparison, vaccinated mice protected from hepatitis retained relatively higher numbers of NKT cells in the liver ($3.6 \pm 0.2\%$ in V β 6 T cell-vaccinated mice and $2.9 \pm 0.7\%$ in V β 10 NKT cell-vaccinated mice). The preservation of liver NKT cells suggests that regulatory CD8⁺ T cells induced by vaccination are capable of controlling the activation of NKT cells.

Regulatory CD8⁺ T cells home toward and proliferate in the liver and the spleen

In concert with the demonstration of the important role of vaccination-induced CD8⁺ T cells, the tracking of fluorescent (CFSE⁺) adoptively transferred CD8⁺ T cells administered i.v. revealed greater survival and proliferation of regulatory CD8⁺ T cells from vaccinated mice, compared with CD8⁺ T cells from control (PBS-treated) mice (Fig. 4A, 4B). As shown above, the relative preservation of liver NKT cells in vaccinated mice upon Con A-injection suggests that regulatory CD8⁺ T cell control might be exerted at the site of inflammation—the liver. Accordingly, we found that CD8⁺ T cells homed toward and proliferated to a large extent in the liver of the protected mice. Interestingly, analysis of the secondary lymphoid organs revealed the presence and survival of CD8⁺ T cells to a larger extent in the spleen and lymph nodes of vaccinated mice compared with control mice (Fig. 4C). Regulatory CD8⁺ T cells that homed to the spleen proliferated to a similar extent as those in the liver, indicating that these

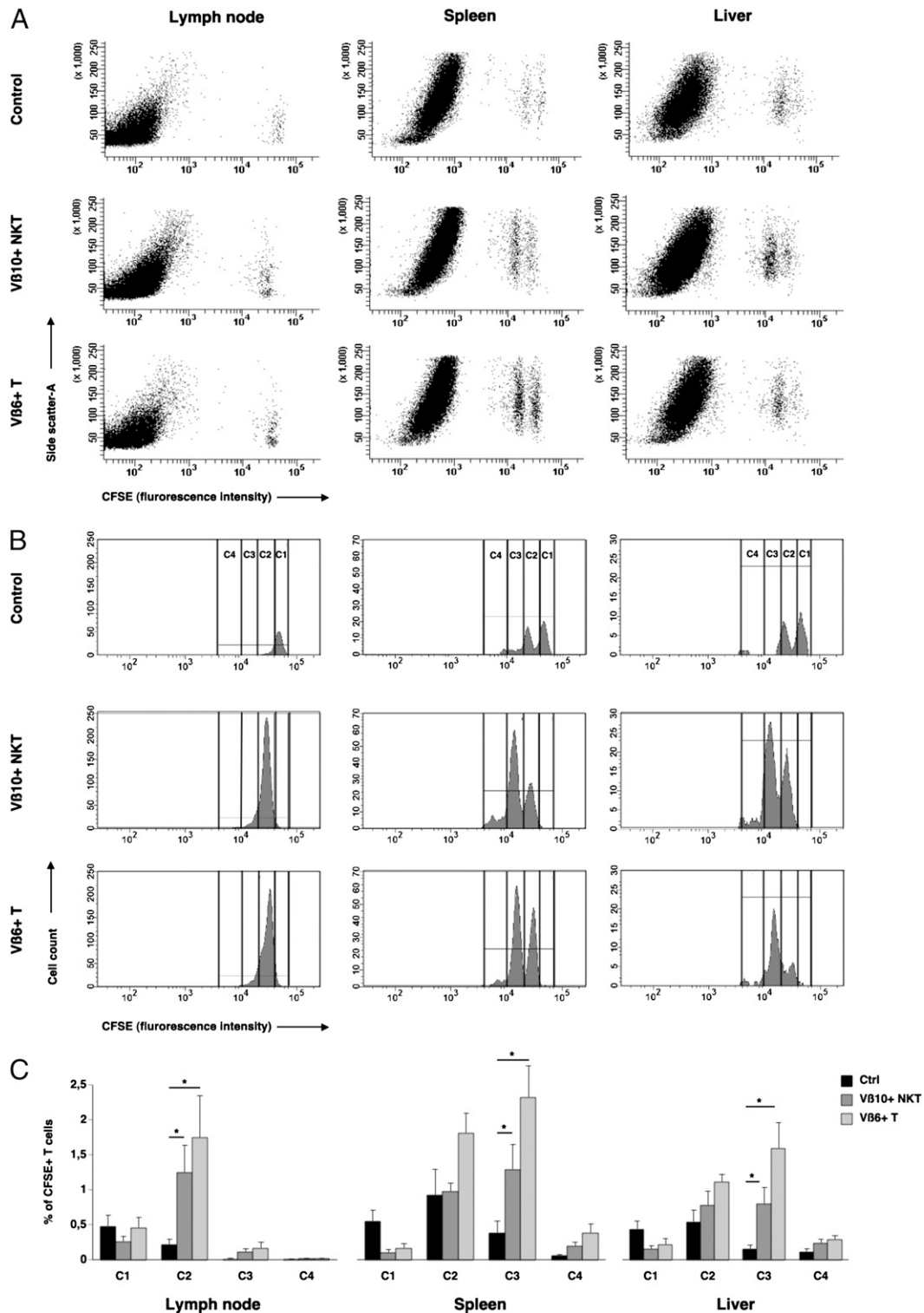


FIGURE 4. Greater extent of survival and site-specific proliferation of protective CD8⁺ T cells. CFSE-labeled CD8⁺ T cells were adoptively transferred from mice vaccinated with $\text{V}\beta 10^+$ NKT ($\text{V}\beta 10^+$ NKT, $n = 5$) or $\text{V}\beta 6^+$ T cell hybridomas ($\text{V}\beta 6^+$ T, $n = 5$) or injected with PBS (Ctrl, $n = 5$), and hepatitis (10 mg/kg ConA) was induced in recipient mice 16 h later. **A**, Representative dot plots gated on total CD8⁺ T cells in the lymph nodes, spleen, and liver of recipient mice 24 h after Con A injection. The figure depicts the side-scatter area on the y-axis and CFSE intensity on the x-axis. **B**, Representative flow cytometric analysis of CFSE⁺ CD8⁺ T cells in the lymph nodes, spleen, and liver of differentially treated recipient mice, 24 h after Con A injection. Figures represent the total cell count (y-axis) as a function of CFSE fluorescence intensity (x-axis). The vertical bars divide CFSE fluorescence intensity into corresponding cell cycles (C1, cells least divided to C4, cells the most divided). **C**, The percentage of CFSE⁺ T cells belonging to the C1, C2, C3, and C4 cycles, among total CD8⁺ T cells in the different organs is represented as mean \pm SEM. *Statistical significance with $p < 0.05$, calculated using the ANOVA Fishers PLSD test (mean \pm SEM).

cells are probably able to control T cell responses in the spleen as well (Fig. 4B). Surprisingly, no damage of splenic tissue was observed upon Con A injection (data not shown). This finding uncovers a strong relationship between the two sites during liver pathology and during vaccination-induced protection. Adoptively transferred regulatory CD8⁺ T cells might thus be capable of exerting control over T cell activation in both the secondary lymphoid organs and the liver, where the pathogenic immune response takes place.

Vaccination-induced regulatory CD8⁺ T cells are restricted to the Qa-1 molecule

The V β -dependent protective responses triggered by TCv procedures have been shown to be Qa-1-dependent—that is, the presentation of V β -derived sequences in the peptide binding groove of the Qa-1 seems to be central in the induction of regulatory CD8⁺ T cells. The fact that the protection we observed was V β -independent raised the question of the Qa-1 dependence in our model. To address this issue, CD8⁺ T cells from wild type C57BL/6J and Qa-1-deficient mice vaccinated with NKT or T cell hybridomas were adoptively transferred to naive wild type recipients, followed by hepatitis induction. Qa-1-deficient mice lack Qa-1-restricted CD8⁺ T cells provoked during TCv (16). As before (Fig. 3), the transfer of CD8⁺ T cells from wild type vaccinated mice protected the recipients from hepatitis (Fig. 5B, left panel). Interestingly, mice that received CD8⁺ T cells from both NKT and T cell-vaccinated Qa-1-deficient mice failed to confer protection to recipients (Fig. 5B, right panel), indicating that, in our model, the V β -independent protection observed is dependent on the presentation of distinct peptides by the Qa-1 molecule that induce Qa-1-restricted CD8⁺ T cells capable of controlling NKT cell-mediated pathology.

Regulatory CD8⁺ T cells are spontaneously induced upon pathologic T cell activation

T cells used as vaccinating cells during TCv need to be activated to be effective (10, 22), suggesting that pathologic activation of endogenous T cells might similarly induce Qa-1-restricted regulatory CD8⁺ T cells. To investigate the spontaneous induction of such a control during pathophysiology, disease was triggered in wild type C57BL/6J mice or Qa-1-deficient mice by the injection of a low dose of Con A. We postulated that Qa-1 on activated T cells in wild type mice, would present peptides similar to those presented by the Qa-1 of vaccinating cells. Three weeks after disease induction, CD8⁺ T cells were purified from the mice and transferred to naive wild type C57BL/6J recipients followed by the induction of hepatitis 16 h later (Fig. 5C). Mice that received CD8⁺ T cells from wild type mice, not subjected to prior ConA treatment, developed fulminant hepatitis (Fig. 5D, left panel). Strikingly, CD8⁺ T cells from mice that had previously developed hepatitis completely protected the recipients from the development of the disease (Fig. 5D, left panel) as efficiently as CD8⁺ T cells induced upon NKTCv (Fig. 5B, left panel). Importantly, CD8⁺ T cells from Con A-injected Qa-1-deficient mice did not confer protection to recipient mice (Fig. 5D, right panel). These mice developed hepatitis to a similar extent as those that received CD8⁺ T cells from naive Qa-1-deficient mice (Fig. 5D, right panel). Thus, similar to TCv, the regulatory CD8⁺ T cells induced by the activation of T cells during pathophysiology are also restricted to Qa-1.

Discussion

The principle characteristic of the TCv approach is its ability to induce regulatory Qa-1-restricted CD8⁺ T cells that are specific for peptides derived from the nonhypervariable region of the TCR (8, 9).

Induction of such regulatory T cells inhibits the activation of CD4⁺ T cells that express similar V β -chains irrespective of their Ag specificity. We tapped into this unique characteristic of TCv to target NKT cells that possess a limited V β repertoire. In an NKT cell-mediated murine disease model, we demonstrate that Qa-1-restricted CD8⁺ T cells induced upon TCv can control NKT cell activation, thereby indicating that TCv could be used as a therapeutic tool in disorders characterized by pathologic NKT cell activation.

The experimental disease model adopted in this study is the induction of NKT cell-mediated hepatitis by the i.v. injection of Con A. Although this disease is dependent on NKT cell activation (19), the systemic administration of Con A entails the polyclonal activation of T cells. In this model, TCv induced regulatory Qa-1-restricted CD8⁺ T cells with specificities independent of peptides derived from V β -chains. Indeed, pioneer studies performed in the 1980s demonstrated that TCv induces regulatory (suppressive) T cells that are either specific for clonotypic molecules (V β epitopes) or for molecules associated with T cell activation but independent of their TCR (23). We propose that TCv performed in models characterized by the oligoclonal activation of T cells as seen in experimental autoimmune encephalomyelitis (24) might favor an anticolonotypic CD8⁺ T cell response, because of the expression of high numbers of identical surface Qa-1-V β complexes. Alternatively, the polyclonal activation of T cells observed in the Con A-induced hepatitis model likely increases the relative numbers of peptides derived from activation-associated molecules bound to Qa-1, thus favoring a regulatory CD8⁺ T cell response capable of controlling a larger population of T cells regardless of their V β expression. The Con A-induced hepatitis model is therefore ideally suited to study this population of regulatory CD8⁺ T cells.

The identical protection observed upon NKTCv and TCv indicates the similarity between NKT cells and conventional T cells in their ability to present peptides in the Qa-1 molecule to specific CD8⁺ T cells, and probably in their repertoire of peptides presented in the Qa-1 molecule. Despite the V β -independence of the protection observed in our model, in NKT cell pathologies that do not involve polyclonal T cell activation, TCv might nevertheless allow us to tap into the unusual characteristic of NKT cells of expressing a limited repertoire of V β -chains, to target NKT cells.

The possibility of mobilizing Qa-1-restricted CD8⁺ T cells upon TCv indicates that these cells are a part of the naive T cell repertoire. Indeed, we demonstrate that the pathophysiologic primary activation of polyclonal T cells by a primary T cell response induces the mobilization and the expansion of this regulatory CD8⁺ T cell population. Such a spontaneous induction of Qa-1-restricted CD8⁺ T cells is an important step in understanding the control of immune responses. As demonstrated in earlier studies, the Qa-1-restricted CD8⁺ T cells do not seem to be implicated in the control of primary immune responses, because these responses are normal in Qa-1-deficient and CD8⁺ T cell-deficient mice (16). However, secondary immune responses involving oligoclonal CD4⁺ T cell activation are exaggerated in these mice (16). These findings are in league with our results, in that the disease incidence during the initial induction of hepatitis in Qa-1-deficient mice is similar to wild type counterparts (data not shown). However, Qa-1-restricted CD8⁺ T cells alone are able to control similar secondary immune responses in distinct hosts. Therefore, this study describes the induction of a regulatory system that might be induced upon each T cell response and that ensures that similar responses are not reactivated immediately despite Ag persistence. Several mechanisms of CD4⁺ T cell control by Qa-1-restricted CD8⁺ T cells can be envisaged. Qa-1-restricted CD8⁺ T cells could directly lyse target T cells as has been

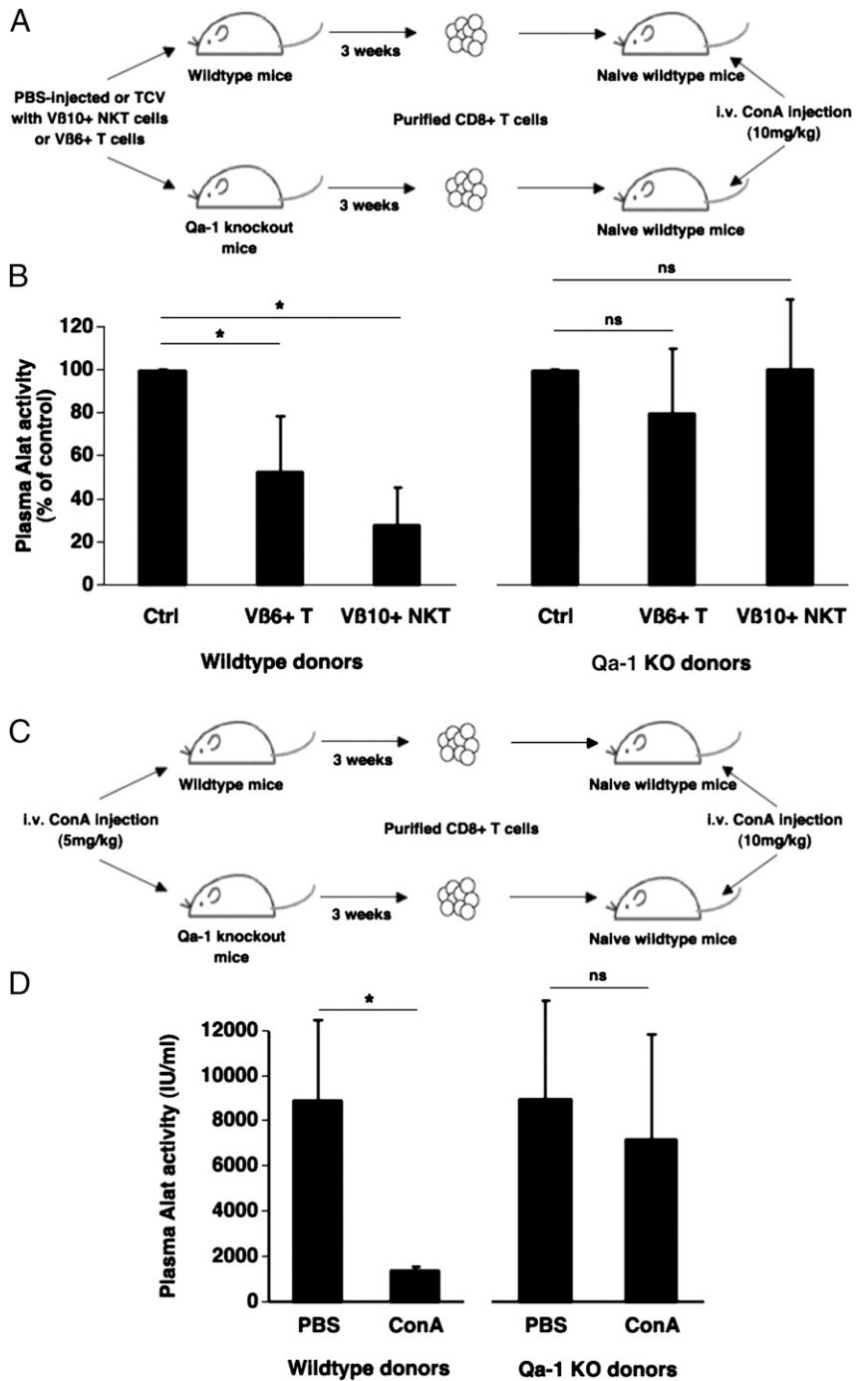


FIGURE 5. NKTCV- and TCV-induced CD8⁺ T cells are restricted to the Qa-1 molecule and can be induced during the pathophysiologic activation of T cells. *A*, Representation of the experimental design; C57BL/6J (wildtype) and Qa-1 knockout mice were vaccinated with Vβ10⁺ NKT (Vβ10⁺ NKT, *n* = 5) or Vβ6⁺ T cell hybridomas (Vβ6⁺ T, *n* = 5) or injected with PBS (Ctrl, *n* = 5). Three weeks later, purified CD8⁺ T cells from the spleens of these mice were adoptively transferred to naive C57BL/6J recipients followed by hepatitis induction 16 h later. Plasma Alar activity was quantified 8 h after disease induction. *B*, The figure represents the percentage of plasma Alar levels compared with the controls of the respective groups. *C*, Representation of the experimental design; C57BL/6J or Qa-1 KO mice were injected with either PBS (*n* = 5) or Con A (5 mg/kg; *n* = 5). Three weeks later, purified CD8⁺ T cells from the spleen were adoptively transferred to naive mice. Sixteen hours after cell transfer, hepatitis was induced by i.v. injection of Con A. *D*, Plasma Alar enzyme activity in wild type (*left panel*) and Qa-1 KO (*right panel*) recipients represented in international units per milliliter was measured 8 h after disease induction. *Statistical significance at *p* < 0.05, calculated using the ANOVA Fishers PLSD test (mean ± SEM). ns, nonsignificant.

proposed in pioneering studies on TCV (25), and/or render APCs incompetent to efficiently activate CD4⁺ T cells as recently reported (26, 27). The detailed description of the molecular mechanisms of regulation will require the isolation of these CD8⁺ T cells, which is something that we are unable to achieve to date.

The key molecule in this regulation, Qa-1, can be thus considered as a tag for the state of activation of the T cells. Qa-1-Qdm complexes are a tag for the functional competence of the cell under nonactivated conditions, protecting it from killer cell-mediated death. However, upon activation, Qdm is replaced by a number of peptides derived from activation-associated molecules that induce specific Qa-1-restricted CD8⁺ T cell activation and expansion. Intriguingly, most peptides presented in the Qa-1 molecule under nonactivated (Qdm peptide) and activated

conditions are derived from the leader sequences of proteins. Leader peptides derived from currently processed proteins might be markers by which the cell is tagged during the immune response. By this mechanism, Qa-1-peptide complexes might leave an imprint of the recent immune response in the form of regulatory CD8⁺ T cells. Therefore, an immediate reactivation of similar T cell clones might be detected by the CD8⁺ T cells and controlled, thus restraining the amplitude of a second reactivation. The persistence of such a system of regulation, however, would be puzzling because it might prevent all future T cell activation. It is thus improbable that such regulatory CD8⁺ T cell responses generate differentiated memory cells.

The ability of suppressor (regulatory) T cells to recognize the activation state of T cells was termed by Cohen et al. in 1989 (23) as

anti-ergotypic response mediated by the recognition of ergotopes (activation-associated molecules). However, the nature of the anti-ergotypic response is still debated. Studies on DNA and peptide vaccinations with potential ergotopes such as the IL-2R β -chain CD25 (28) and the stress-inducible heat shock protein 60 (29) show varied anti-ergotypic responses that are mediated by $\alpha\beta$ or $\gamma\delta$ TCR⁺ T cells that are restricted to the MHC class I or II molecules (30). The subset of Qa-1-restricted CD8⁺ T cells revealed in our study might therefore be part of a larger anti-ergotypic regulatory system that tightly regulates T cell immune responses. In our model, Con A-induced polyclonal activation of T cells may lead to the presentation of peptides derived from more than one ergotope by Qa-1. The definition of these ergotopes is, however, beyond the scope of this study.

The illustration of the existence of such regulatory T cells capable of controlling polyclonal T cell activation opens a new field of research in T cell regulation. The in-depth study of these cells will enable better comprehension of T cell reactivation and the therapeutic potential of these regulatory T cells in the suppression of diseases characterized by polyclonal T cell activation. Finally, preventing their induction might be a way to potentiate vaccines.

Acknowledgments

We thank Dr. Mitchell Kronenberg (La Jolla Institute of Allergy and Immunology) for providing NKT cell hybridomas; the National Institutes of Health Tetramer facility for providing CD1d- α -galcer tetramers; and the IFR02 Biochemical services, Faculty of Medicine Xavier Bichat, for the biochemical analysis of hepatic enzymes.

Disclosures

The authors have no financial conflicts of interest.

References

- Walker, L. S., and A. K. Abbas. 2002. The enemy within: keeping self-reactive T cells at bay in the periphery. *Nat. Rev. Immunol.* 2: 11–19.
- Aldrich, C. J., A. DeCloux, A. S. Woods, R. J. Cotter, M. J. Soloski, and J. Forman. 1994. Identification of a Tap-dependent leader peptide recognized by alloreactive T cells specific for a class Ib antigen. *Cell* 79: 649–658.
- Moser, J. M., J. Gibbs, P. E. Jensen, and A. E. Lukacher. 2002. CD94-NKG2A receptors regulate antiviral CD8⁺ T cell responses. *Nat. Immunol.* 3: 189–195.
- Vance, R. E., J. R. Kraft, J. D. Altman, P. E. Jensen, and D. H. Raulet. 1998. Mouse CD94/NKG2A is a natural killer cell receptor for the nonclassical major histocompatibility complex (MHC) class I molecule Qa-1(b). *J. Exp. Med.* 188: 1841–1848.
- Tompkins, S. M., J. R. Kraft, C. T. Dao, M. J. Soloski, and P. E. Jensen. 1998. Transporters associated with antigen processing (TAP)-independent presentation of soluble insulin to alpha/beta T cells by the class Ib gene product, Qa-1(b). *J. Exp. Med.* 188: 961–971.
- Davies, A., S. Kalb, B. Liang, C. J. Aldrich, F. A. Lemonnier, H. Jiang, R. Cotter, and M. J. Soloski. 2003. A peptide from heat shock protein 60 is the dominant peptide bound to Qa-1 in the absence of the MHC class Ia leader sequence peptide Qdm. *J. Immunol.* 170: 5027–5033.
- Lo, W. F., A. S. Woods, A. DeCloux, R. J. Cotter, E. S. Metcalf, and M. J. Soloski. 2000. Molecular mimicry mediated by MHC class Ib molecules after infection with gram-negative pathogens. *Nat. Med.* 6: 215–218.
- Jiang, H., H. Kashleva, L. X. Xu, J. Forman, L. Flaherty, B. Pernis, N. S. Braunstein, and L. Chess. 1998. T cell vaccination induces T cell receptor Vbeta-specific Qa-1-restricted regulatory CD8⁺ T cells. *Proc. Natl. Acad. Sci. USA* 95: 4533–4537.
- Li, J., I. Goldstein, E. Glickman-Nir, H. Jiang, and L. Chess. 2001. Induction of TCR Vbeta-specific CD8⁺ CTLs by TCR Vbeta-derived peptides bound to HLA-E. *J. Immunol.* 167: 3800–3808.
- Ben-Nun, A., H. Wekerle, and I. R. Cohen. 1981. Vaccination against autoimmune encephalomyelitis with T-lymphocyte line cells reactive against myelin basic protein. *Nature* 292: 60–61.
- Panoutsakopoulou, V., K. M. Huster, N. McCarty, E. Feinberg, R. Wang, K. W. Wucherpfennig, and H. Cantor. 2004. Suppression of autoimmune disease after vaccination with autoreactive T cells that express Qa-1 peptide complexes. *J. Clin. Invest.* 113: 1218–1224.
- Tang, X., I. Maricic, N. Purohit, B. Bakamjian, L. M. Reed-Loisel, T. Beeston, P. Jensen, and V. Kumar. 2006. Regulation of immunity by a novel population of Qa-1-restricted CD8alphaalpha+TCRalphabeta+ T cells. *J. Immunol.* 177: 7645–7655.
- Kumar, V., R. Tabibiazar, H. M. Geysen, and E. Sercarz. 1995. Immunodominant framework region 3 peptide from TCR V beta 8.2 chain controls murine experimental autoimmune encephalomyelitis. *J. Immunol.* 154: 1941–1950.
- Wilson, S. B., and T. L. Delovitch. 2003. Janus-like role of regulatory iNKT cells in autoimmune disease and tumour immunity. *Natl. Rev. Immunol.* 3: 211–222.
- Griseri, T., L. Beaudoin, J. Novak, L. T. Mars, F. Lepault, R. Liblau, and A. Lehuen. 2005. Invariant NKT cells exacerbate type 1 diabetes induced by CD8 T cells. *J. Immunol.* 175: 2091–2101.
- Hu, D., K. Ikizawa, L. Lu, M. E. Sanchirico, M. L. Shinohara, and H. Cantor. 2004. Analysis of regulatory CD8 T cells in Qa-1-deficient mice. *Nat. Immunol.* 5: 516–523.
- Brossay, L., S. Tangri, M. Bix, S. Cardell, R. Locksley, and M. Kronenberg. 1998. Mouse CD1-autoreactive T cells have diverse patterns of reactivity to CD1+ targets. *J. Immunol.* 160: 3681–3688.
- Harris, J. F., A. F. Chambers, and A. S. Tam. 1991. Modulation of clonal progression in B16F1 melanoma cells. *Clin. Exp. Metastasis* 9: 151–162.
- Takeda, K., Y. Hayakawa, L. Van Kaer, H. Matsuda, H. Yagita, and K. Okumura. 2000. Critical contribution of liver natural killer T cells to a murine model of hepatitis. *Proc. Natl. Acad. Sci. USA* 97: 5498–5503.
- Tiegs, G., J. Hentschel, and A. Wendel. 1992. A T cell-dependent experimental liver injury in mice inducible by concanavalin A. *J. Clin. Invest.* 90: 196–203.
- Song, E., S. K. Lee, J. Wang, N. Ince, N. Ouyang, J. Min, J. Chen, P. Shankar, and J. Lieberman. 2003. RNA interference targeting Fas protects mice from fulminant hepatitis. *Nat. Med.* 9: 347–351.
- Lider, O., M. Shinitzky, and I. R. Cohen. 1986. Vaccination against experimental autoimmune diseases using T lymphocytes treated with hydrostatic pressure. *Ann. N. Y. Acad. Sci.* 475: 267–273.
- Lohse, A. W., F. Mor, N. Karin, and I. R. Cohen. 1989. Control of experimental autoimmune encephalomyelitis by T cells responding to activated T cells. *Science* 244: 820–822.
- Fazilleau, N., C. Delarasse, C. H. Sweenie, S. M. Anderton, S. Fillatreau, F. A. Lemonnier, D. Pham-Dinh, and J. M. Kanellopoulos. 2006. Persistence of autoreactive myelin oligodendrocyte glycoprotein (MOG)-specific T cell repertoires in MOG-expressing mice. *Eur. J. Immunol.* 36: 533–543.
- Jiang, H., R. Ware, A. Stall, L. Flaherty, L. Chess, and B. Pernis. 1995. Murine CD8⁺ T cells that specifically delete autologous CD4⁺ T cells expressing V beta 8 TCR: a role of the Qa-1 molecule. *Immunity* 2: 185–194.
- Jiang, H., X. Tang, I. Maricic, Z. Garcia, S. Fanchiang, and V. Kumar. 2009. Dendritic cells use endocytic pathway for cross-priming class Ib MHC-restricted CD8alphaalpha+TCRalphabeta+ T cells with regulatory properties. *J. Immunol.* 182: 6959–6968.
- Smith, T. R., and V. Kumar. 2008. Revival of CD8⁺ Treg-mediated suppression. *Trends Immunol.* 29: 337–342.
- Mimran, A., F. Mor, P. Carmi, F. J. Quintana, V. Rotter, and I. R. Cohen. 2004. DNA vaccination with CD25 protects rats from adjuvant arthritis and induces an anti-ergotypic response. *J. Clin. Invest.* 113: 924–932.
- Quintana, F. J., A. Mimran, P. Carmi, F. Mor, and I. R. Cohen. 2008. HSP60 as a target of anti-ergotypic regulatory T cells. *PLoS ONE* 3: e4026.
- Mimran, A., F. Mor, F. J. Quintana, and I. R. Cohen. 2005. Anti-ergotypic T cells in naïve rats. *J. Autoimmun.* 24: 191–201.