Ubiquitous antigen-specific T regulatory type 1 cells variably suppress hepatic and extrahepatic autoimmunity

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Peptide MHC class II–based (pMHCII-based) nanomedicines trigger the formation of multicellular regulatory networks by reprogramming autoantigen-experienced CD4⁺ T cells into autoimmune disease-suppressing T regulatory type 1 (TR1) cells. We have shown that pMHCII-based nanomedicines displaying liver autoimmune disease–relevant yet ubiquitously expressed antigens can blunt various liver autoimmune disorders in a non–disease–specific manner without suppressing local or systemic immunity against infectious agents or cancer. Here, we show that such ubiquitous autoantigen-specific T cells are also awakened by extrahepatic tissue damage and that the corresponding TR1 progeny can suppress experimental autoimmune encephalomyelitis (EAE) and pancreatic β cell autoreactivity. In mice having EAE, nanomedicines displaying either ubiquitous or CNS-specific epitopes triggered the formation and expansion of cognate TR1 cells and their recruitment to the CNS-draining lymph nodes, sparing their liver-draining counterparts. Surprisingly, in mice having both liver autoimmunity and EAE, liver inflammation sequestered these ubiquitous or even CNS-specific TR1 cells away from the CNS, abrogating their antiencephalitogenic activity. In these mice, only the ubiquitous antigen-specific TR1 cells suppressed liver autoimmunity. Thus, the scope of antigen spreading in autoimmune disorders is larger than previously anticipated, involving specificities expected to be silenced by mechanisms of tolerance; the regulatory activity, but not the retention of autoreactive TR1 cells, requires local autoantigen expression.

Introduction

A growing body of evidence has established the feasibility of using antigen-specific approaches for the treatment of autoimmunity (1). We have shown that nanoparticles (NPs) coated with disease-relevant peptide MHC (pMHC) molecules (2) can resolve inflammation in various organ-specific autoimmune disease models in a disease-specific manner without impairing normal immunity (3–5). In all these models, pMHC class II-NP (pMHCII-NP) therapy functions by reprogramming cognate autoantigen-experienced CD4⁺ T cells into FoxP3–CD25– T regulatory type 1-like (TR1-like) cells, followed by systemic expansion. When these cells encounter costimulation-competent autoantigen-presenting cells (APCs) in the target organ and proximal lymphoid tissues, they produce regulatory cytokines, including IL-10, TGF-β, and IL-21, leading to comprehensive inhibition of autoreactive T cell activation and recruitment and disease reversal.

NOD.c3c4 mice are resistant to T1D but develop a form of autoimmune biliary disease that resembles human primary biliary cholangitis (PBC) (6). Like human PBC, PBC in NOD.c3c4 mice is associated with spontaneous T and B cell responses against the mitochondrial pyruvate dehydrogenase (PDC) complex (7). Treatment of NOD.c3c4 mice with NPs displaying IA⁺B₃₂₅₄ presenting mPDC-E2 epitopes blunted the progression of, and reversed overt, PBC (5). In contrast, treatment with NPs coated with the pancreatic β cell–specific BDC₂.₅m/IA⁺B₃₂₅₄ pMHC triggered neither TR1 cell expansion nor disease reversal. This outcome was consistent with the fact that pMHCII-NPs exclusively operate on autoantigen-experienced CD4⁺ T cells (4).

Since PDC is an autoantigen expressed in virtually all cell types, our results raised the question of whether PBC-relevant nanomedicines were disease specific. This was addressed by investigating their pharmacodynamic activity in models of primary sclerosing cholangitis (PSC) and autoimmune hepatitis (AIH). We reasoned that bile duct or hepatocyte damage in PBC and PSC or AIH, respectively, would trigger the release of not only the PBC-relevant autoantigen PDC, but also the AIH-relevant autoantigens cytochrome P450 (CYP2D6) and formimido-ytransferase cyclochrome (FTCD), leading to the priming of autoreactive CD4⁺ T cells capable of responding to the corresponding pMHC-NPs. This was indeed the case (5). Remarkably, these therapeutic effects were dissociated from impairment of normal immunity (5).

The current study was initiated to investigate the following: (a) whether spontaneous or induced extrahepatic cell death can trigger the activation of ubiquitous antigen-specific CD4⁺ T
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BDC 2.5mi/IA^7-NPs triggered the formation of cognate TR1-like CD4+ T cells and their accumulation in the LNs draining both liver and pancreas (portal/celiac LNs [PCLNs] and pancreatic LNs [PLNs]), but not in nondraining LNs (mesenteric LNs [MLNs]) or in the liver (Figure 1A and Supplemental Figure 1, C and D). This suggested that spontaneous β cell killing in prediabetic NOD mice does not trigger the formation of PDC-E2 or CYPD2D6 autoantigen–experienced T cells capable of responding to pMHCII-NPs. We entertained 3 alternative possibilities to explain these results: (a) these antigens are released from cholangiocytes and hepatocytes (in liver autoimmunity), but not from dying β cells (in type 1 diabetes [T1D]); (b) the antigens are released from β cells, but NOD mice do not export cognate autoreactive T cells for these antigens; or (c) the antigens are released and the mice harbor cognate T cells, but the antigens are released in insufficient amounts.

cells and thus render these cells responsive to the reprogramming properties of pMHC-based nanomedicines and (b) whether the pharmacodynamic and therapeutic effects of these nanomedicines, displaying ubiquitous antigenic epitopes, are liver specific or also able to suppress extrahepatic autoimmunity as compared with pMHC-based nanomedicines displaying tissue-specific or irrelevant antigens.

Results and Discussion

Treatment of 10-week-old NOD mice with PDC-E2_{166-181}/IA^7-NPs (PBC relevant) and CYPD_{398-412}/IA^7-NPs (AIH relevant) did not trigger the expansion of cognate TRI-like CD4+ T cells relative to endogenous BDC_{2.5mi}/IA^7-specific T cells from untreated mice in blood or other organs, including liver (Supplemental Figure 1, A and B; supplemental material available online with this article; https://doi.org/10.1172/JCI130670DS1). As expected, BDC_{2.5mi}/IA^7-NPs triggered the formation of cognate TRI-like CD4+ T cells and their accumulation in the LNs draining both liver and pancreas (portal/ceelial LNs [PCLNs] and pancreatic LNs [PLNs]), but not in nondraining LNs (mesenteric LNs [MLNs]) or in the liver (Figure 1A and Supplemental Figure 1, C and D). This suggested that spontaneous β cell killing in prediabetic NOD mice does not trigger the formation of PDC-E2 or CYPD2D6 autoantigen–experienced T cells capable of responding to pMHCII-NPs. We entertained 3 alternative possibilities to explain these results: (a) these antigens are released from cholangiocytes and hepatocytes (in liver autoimmunity), but not from dying β cells (in type 1 diabetes [T1D]); (b) the antigens are released from β cells, but NOD mice do not export cognate autoreactive T cells for these antigens; or (c) the antigens are released and the mice harbor cognate T cells, but the antigens are released in insufficient amounts.
To distinguish among these possibilities, we expressed an X chromosome-linked rat-insulin promoter-driven human diphtheria toxin (DT) receptor (hDTR) transgene in NOD mice and administered DT to kill about 50% of β cells (due to X chromosome inactivation, only 50% express the hDTR) (8). We then gave DT-treated mice PDC-E2 166–181/IAg7–NPs, CYPD 398–412/IAg7–NPs (PBC/AIH relevant), BDC 2.5mi/IAg7–NPs (T1D-specific), MOG 36–50/IAg7–NPs (experimental autoimmune encephalomyelitis specific [EAE-specific]) or Cys-coated NPs (control). As expected, MOG 36–50/IAg7–NPs did not expand cognate tetramer+ CD4+ T cells (Figure 1B). In contrast, both PDC-E2 166–181/IAg7– and CYPD 398–412/IAg7–NPs triggered the formation and accumulation of cognate T cells expressing the TR1 markers LAG3, LAR, and CD49b in the spleen and PLNs, to an extent similar to that seen for BDC 2.5mi/IAg7–NP–treated animals (Figure 1B and Supplemental Figure 1, E and F). Furthermore, whereas the PCLNs and PLNs of NOD.RIP-hDTR mice treated with DT plus Cys-NPs supported the proliferation of exogenous CFSE-labeled IGRP206–214–specific CD8+ T cells (upon recognition of β cell–derived IGRP draining into these LNs), neither the PCLNs nor PLNs of NOD.RIP-hDTR mice treated with DT plus PDC-E2166–181/IAg7–NPs could do so (Figure 1, C and D). These observations support the third scenario described earlier and demonstrate that the PDC-E2166–181/IAg7–NP–induced TR1 cells that accumulate in the PCLNs/PLNs suppress the activation of noncognate β cell–autoreactive T cells by local APCs (loaded with β cell–derived PDC-E2 and IGRP). In addition, these findings corroborate that pMHC-NP–induced TR1 cell formation requires autoantigen-experienced T cells (absent in non-DT-treated mice) (Figure 1, A–D and Supplemental Figure 1, A).
nate TR1-like cells in the CLNs (Figure 2A). These effects were also seen in C57BL/6 mice with EAE that received PDC-E2 94–108/IAb–NPs or CYPD 353–367/IAb–NPs (Figure 2, D and E; Supplemen-
chal Figure 2, A–C; and Supplemental Figure 3A). Thus, upon oli-
godendrocyte damage, both PDC-E2 and CYPD2D6 are deliv-
ered to proximal APCs for autoreactive CD4+ T cell priming,
enabling TR1 cell generation by pMHC-NPs, recruitment to the
CLNs, and suppression of EAE.

We next asked whether accumulation of pMHCII-NP–induced
TR1 cells is driven by inflammation in a non–antigen- specific
manner. We tracked MOG 38–49/IAb–, PDC-E2 94–108/IAb–, and
CYPD353–367/IAb–specific TR1 cells arising in pMHCII-NP–treat-
ed B6 mice having both AIH and EAE (Supplemental Figure
4A). MOG 38–49/IAb–NPs triggered expansion and accumulation
of cognate TR1-like CD4+ T cells in blood, spleen, and CLNs, but
not in the liver, PCLNs, or MLNs (Supplemental Figure 5, A–C).
Mice treated with Fla 462–472/IAb–NPs, displaying a colitis-relevant
gut microbial epitope, lacked both MOG 38–49/IAb– and Fla 462–472/
and B). Furthermore, they indicate that NOD mice harbor T cells
targeting ubiquitously expressed antigens and that the priming
of such cells requires antigen shedding.

We next asked whether the therapeutic effects of these ubiq-
utous antigen-based nanomedicines were liver specific. We
compared the ability of PDC-E2 166–181/IAg7–NPs and CYPD 398–412/
IAg7–NPs vs. BDC 2.5mi/IAg7–NPs and MOG 36–50/IAg7–NPs to blunt
MOG36–55–induced EAE in NOD mice; disease kinetics/severi-
ity were similar to those reported earlier (9). BDC 2.5mi/IAg7–NPs
expanded cognate TR1-like T cells as in non–EAE-affected NOD
mice (4), but these cells were absent from the CNS-draining cer-
vical LNs (CLNs) and liver (Figure 2A and Supplemental Figure
1, G–I) and had no antiencephalitogenic activity (Figure 2, B and
C; and Supplemental Figure 1J). Notably, both CYPD 398–412/IAg7–
NPs and PDC-E2 166–181/IAg7–NPs also triggered TR1 cell expan-
sion (Figure 2A and Supplemental Figure 1, G–I), but unlike
BDC 2.5mi/IAg7–NPs, suppressed EAE (Figure 2, B and C; and Sup-
plemental Figure 1J) in association with accumulation of cog-

Figure 3. In mice with both EAE and AIH, ubiquitous antigen-specific TR1 cells are selectively recruited to the liver. (A) EAE scores are shown: 11 Fla462–472/IAb–NP, 7 MOG38–49/IAb–NP, 10 PDC94–108/IAb–NP, and 7 CYPD353–367/IAb–NP. (B) Average rank order LFB scores. Data correspond to n = 9, 9, 6, and 6 mice (left to right). (C) Average histopathological scores from the mice shown in A. Data correspond to n = 11, 10, 7, and 7 mice (left to right). (D) Serum ALT levels: n = 11, 10, 7, and 7 mice (left to right). (E) Serum ALT levels in B6 mice with AIH receiving Cys-NPs (n = 24) or PDC-E2 94–108/IAb–NPs (n = 24). (F) Average EAE scores in B6 mice with AIH treated with Cys-NPs pre-EAE, or Cys-NPs (n = 6), PDC-E2 94–108/IAb–NPs (n = 7), or MOG 38–49/IAb–NPs (n = 7) after EAE induction. (G) EAE incidence (left) and average EAE scores (right) of B6 mice having AIH that received Cys-NPs (n = 24) or PDC-E2 94–108/IAb–NPs (n = 24) before EAE. (H) Therapeutic effect of PDC-E2 94–108/IAb–NPs (n = 5) versus Cys-NPs (n = 3) in PDC-E2 94–108/IAb–NP–treated B6 mice with AIH, after EAE induction. Data correspond to mean ± SEM values. P values were calculated using 2-way ANOVA (A, F, G [right panel], and H), Kaplan-Meier survival (G [left panel]), and 1-way ANOVA with Tukey’s post hoc correction (B, C, and D).

*P < 0.05; **P < 0.01; and ****P < 0.0001.
like cells to the liver was antigen driven; treatment of these mice with BDC2.5mi/IAg7–NPs triggered cognate TR1 cell recruitment to the PLNs and PCLNs, but not to the CLNs, MLNs, or liver (Supplemental Figure 5, E–G). Thus, accumulation of pMHC-NP–induced TR1 cells to sites of inflammation and draining lymphoid tissue and the ensuing therapeutic effects require local autoantigen expression. Whereas this is also true for TR1 cells recognizing ubiquitous antigens, liver inflammation sequesters these cells away from the CNS.

To further probe the role of active liver inflammation in the sequestration of ubiquitous antigen-specific TR1-like cells, we tracked their recruitment in mice in which EAE was induced upon treatment of AIH (Supplemental Figure 4B). As expected, PDC-E294–108/IAb–NPs, but not Fla462–472 /IAb–NPs, reversed EAE (Figure 3, A and B; and Supplemental Figure 3B) without suppressing AIH (Figure 3, C and D; and Supplemental Figure 5D).

Surprisingly, treatment with PDC-E294–108/IAb– or CYPD353–367/IAb–NPs, which in EAE mice without AIH led to TR1 cell recruitment to the CLNs and EAE reversal (Figure 2, D and E; and Supplemental Figure 2A), resulted in the accumulation of these cells in the liver and liver-draining LNs but not in the CLNs (Supplemental Figure 5, A–C). As a result, PDC-E294–108/IAb–NPs and CYPD353–367/IAb–NPs suppressed liver disease (Figure 3, C and D; and Supplemental Figure 5D), but not EAE (Figure 3, A and B). Additional experiments in a NOD model of AIH (Ad-hFTCD induced) confirmed that recruitment of such ubiquitous antigen-reactive TR1-like cells to the liver was antigen driven; treatment of these mice with BDC2.5mi/IAg7–NPs triggered cognate TR1 cell recruitment to the PLNs and PCLNs, but not to the CLNs, MLNs, or liver (Supplemental Figure 5, E–G). Thus, accumulation of pMHC-NP–induced TR1 cells to sites of inflammation and draining lymphoid tissue and the ensuing therapeutic effects require local autoantigen expression. Whereas this is also true for TR1 cells recognizing ubiquitous antigens, liver inflammation sequesters these cells away from the CNS.

To further probe the role of active liver inflammation in the sequestration of ubiquitous antigen-specific TR1-like cells, we tracked their recruitment in mice in which EAE was induced upon treatment of AIH (Supplemental Figure 4B). As expected, PDC-E294–108/IAb–NPs reduced serum ALT levels versus what was
found in Cys–NP-treated controls (Figure 3E). We then immunized mice having treated or untreated AIH with MOG36–55 to induce EAE. Treatment of the mice that received Cys-NPs after AIH induction with PDC-E294–108/IAb–NPs triggered the recruitment of PDC-E294–108/IAb–reactive CD4+ T cells to the CLNs and liver, but not to the CLNs (as compared with mice that continued to receive Cys-NPs after AIH-induction; Supplemental Figure 5H), and failed to reverse EAE (Figure 3F and Supplemental Figure 3C). In contrast, treatment with MOG38–49/IAb–NPs triggered the recruitment of cognate CD4+ T cells to the CLNs, but not PCLNs or liver, and reversed EAE (Figure 3F, Supplemental Figure 3C, and Supplemental Figure 5H). Only treatment with PDC-E294–108/IAb–NP, but not Cys-NP or MOG38–49/IAb–NP, reduced liver pathology (Supplemental Figure 5I). Thus, continued liver inflammation recruits and retains PDC-E294–108/IAb–specific but not MOG38–49/IAb–specific TR1 cells.

Remarkably, the mice that received PDC-E294–108/IAb–NPs after AIH induction were resistant to EAE (Figure 3G and Supplemental Figure 3D). Furthermore, both the mice that did not develop EAE and those that received PDC-E294–108/IAb–NPs after developing EAE had larger accumulations of cognate CD4+ T cells in the CLNs (as well as PCLNs) than the mice that received Cys-NPs after EAE induction (Supplemental Figure 5H), consistent with the EAE resistance of the former and therapeutic responsiveness of the latter to PDC-E294–108/IAb–NPs (Figure 3H, Supplemental Figure 3E, and Supplemental Figure 5J). Both types of mice had improved liver pathology versus those that received Cys-NP after EAE induction or Cys-NPs after AIH induction (Supplemental Figure 5F). Thus, resolution of liver inflammation releases PDC-E294–108/IAb–specific TR1-like cells for recruitment to the CLNs, enabling them to blunt EAE.

We next superimposed EAE onto the more aggressive, chronic form of liver autoimmunity that develops in NOD.c3c4 mice (Supplemental Figure 4C) (5). The severity of EAE in NOD.c3c4 mice was greater than in NOD mice, suggesting that liver inflammation does not nonspecifically sequester effector CNS-autoreactive CD4+ T cells. We treated these mice with PDC-E2166–182/IAb–NPs, CYPD398–412/IAb–NPs, MOG36–50/IAb–NPs, BDC2.5mi/IAb–NPs, or Cys-NPs. Surprisingly, none of these nanomedicines suppressed the progression of EAE (Figure 4A; Supplemental Figure 3F). The cognate TR1-like CD4+ T cells induced by these nanomedicines were present in the liver and PCLNs, but not in the CLNs or MLNs (Supplemental Figure 6, A–C). Both the splenic and liver-associated MOG36–50/IAb– and PDC-E2166–182/IAb–specific CD4+ T cells of these mice were TR1-like because they coexpressed LAG-3 and CD49b (Supplemental Figure 6, D and E) and produced IL-10 in response to TCR ligation ex vivo, unlike their unstimulated counterparts or stimulated tetramer CD4+ T cells from the same mice (Figure 4, B and C).

Recruitment of the PDC-E2166–182/IAb– and CYPD398–412/IAb–specific TR1-like CD4+ T cells to the liver and PCLNs was associated with improved liver pathology (Figure 4, D and E; and Supplemental Figure 6, F–H). In contrast, although MOG36–50/IAb–NP–induced TR1 CD4+ T cells were recruited to the liver and PCLNs, they did not suppress liver disease, indicating that their immunoregulatory effects require local autoantigen expression (Figure 4, D and E; and Supplemental Figure 6, F–H). Thus, severe liver inflammation can efficiently retain antigen-specific TR1 cells nonspecifically. The larger size, hence higher antigen load, of the liver versus the CNS coupled to the fenestrated liver vasculature may underlie the preferential recruitment of ubiquitous autoantigen-specific TR1 cells to this organ upon inflammation. Additionally, our data indicate that the degree of inflammation (i.e., PBC vs. AIH) and the magnitude of antigen shedding (i.e., spontaneous vs. DT induced) also play important roles. Interestingly, pMHCII-NP–induced TR1 cells upregulate CCR5 and CXCR3 and downregulate CCR7 mRNA as compared with conventional CD4+ T cells (our unpublished data). Since portal vessel–derived CCR5 ligands and liver sinusoid–derived CXCR3 ligands have been implicated in lymphocyte recruitment to the liver, and sinusoidal and lymphatic vessel–derived CCR7 ligands in lymphocyte egress from the liver (10), differential expression of these chemokine receptors may also play a role. Future studies will be needed to determine whether sequestration of ubiquitous autoantigen–specific TR1 cells in the liver will also occur in mice in which liver autoimmunity develops after extrahepatic autoimmunity or whether these phenomena are applicable to disease/organ pairs other than the liver/CNS axis.

Collectively, our results show that pMHCII-based nanomedicines displaying ubiquitous antigenic epitopes can blunt not only various liver autoimmune disorders, but also CNS-specific autoimmunity, albeit not as efficiently as nanomedicines displaying CNS-specific autoantigens. Importantly, our work exposes a biological phenomenon whereby autoreactive T cells against ubiquitous antigens (11) are awakened by antigen shedding from different cells/tissues, including hepatocytes, cholangiocytes, pancreatic β cells, and oligodendrocytes, rendering them responsive to pMHCII-based nanomedicines. This implies that central and peripheral mechanisms of tolerance for systemically expressed autoantigens such as PDC-E2 and CYPD2D6, including clonal deletion and functional inactivation, are not fully penetrant, even in nonautoimmune-prone genetic backgrounds. In turn, this implies that the scope of epitope- and antigen-spreading in autoimmune disorders is much larger than previously anticipated. Furthermore, our observations not only highlight the essential role for local autoantigen expression in the regulatory activity of antigen-specific TR1-like cells but also indicate that liver inflammation has the potential to nonspecifically draw T regulatory cells away from sites of cognate autoantigen expression and autoimmune inflammation (Supplemental Table 1).

Methods

The methods are described in the Supplemental Methods.

Study approval. These studies were approved by the animal care committee of the Cumming School of Medicine.

Author contributions

CSU produced the pMHCs and executed most of the experiments shown in Figures 1–4 and Supplemental Figures 1–6 with contributions from JM, SS, SM, JAL, JY, RHN, KS, UC, YY, and KKE and contributed to writing the manuscript with PS. PS designed the study, supervised and coordinated its execution, and wrote the manuscript with CSU.
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