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Sympathetic T Cells in Rheumatoid Arthritis Resist IDO-Mediated Inhibition

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A hallmark of T cell-mediated autoimmunity is the persistence of autoreactive T cells. However, it remains to elucidate the manner in which synovial T cells are sustained in patients with rheumatoid arthritis (RA). We found that dendritic cells (DC) and tissues from the synovial joints of RA patients expressed higher levels of IDO than DC from healthy donors. Interestingly, T cells derived from the joint synovial fluid (SF) of RA patients proliferated in response to either autologous or allogeneic IDO-positive DC, an outcome that was not affected by the addition of IDO inhibitor 1-methyl-D-tryptophan (1-MT). In contrast, addition of 1-MT to the culture stimulated with allogeneic or autologous IDO-positive DC significantly enhanced the proliferation of T cells derived from peripheral blood of healthy donors or from peripheral blood of RA patients. Furthermore, we found that functionally active tryptophanyl-tRNA-synthetase (TTS) was significantly elevated in T cells derived from the SF of RA patients, leading to enhanced storage of tryptophan in T cells and to subsequent resistance to IDO-mediated deprivation of tryptophan. The RA SF enhancement of TTS expression in T cells was blocked by mAb to IFN-γ and TNF-α. These results suggest that the resistance of T cells to IDO-mediated deprivation of tryptophan represents a mechanism by which autoreactive T cells are sustained in vivo in RA patients. Specifically, blocking of the up-regulation of TTS expression in T cells presents an avenue for development of a novel therapeutic approach to treatment of RA. The Journal of Immunology, 2006, 177: 8226–8233.

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Abbreviations used in this paper: RA, rheumatoid arthritis; 1-MT, 1-methyl-D-tryptophan; C57, cycle threshold; DC, dendritic cell; PB, peripheral blood; PI, propidium iodide; SF, synovial fluid; ST, synovial tissue; TTS, tryptophanyl-tRNA-synthetase.

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in RA patients. We found that RA patient-derived SF contained inflammatory factors (e.g., IFN-γ and TNF-α). These cytokines were able to enhance the expression of TTS in T cells, leading to the resistance of RA patient-derived SF T cells to IDO-mediated deprivation of tryptophan. This means that blocking of TTS may prove valuable for further development of novel approaches to the restoration of IDO-mediated immune tolerance and the diminishing of autoreactive T cell-mediated chronic immune responses.

**Materials and Methods**

**Patient samples**

A group of 35 patients with definitive RA (based on the criteria of the American Rheumatism Association) was investigated in this study, which included 24 females and 11 males whose ages ranged from 24 to 73 years (mean age, 43 years), with a disease duration of 9 ± 6 years. Erythrocyte sedimentation rate, C-reactive protein, and rheumatoid factors were 56.4 ± 20.6 mm/h, 38.0 ± 23.7 mg/L, and 99.8 ± 88.2 IU/mL, respectively. An age-matched group of 36 healthy individuals was included as a control. Peripheral blood (PB) specimens were collected from RA patients or healthy donors, and SF from RA patients during routine knee joint aspirations. Samples of synovial tissue (ST) were obtained from RA patients during wrist synovectomy or arthroplasty. Control ST was obtained from patients with traumatic lesions during knee arthroscopy. No immunosuppressive or immunomodulatory drugs had been taken by patients for at least 3 mo when samples were collected. Informed consent was obtained from all subjects before sample collection. The study protocols were approved by the institutional review board of the Institute of Health Sciences (Shanghai, China).

**Cell preparation**

Rheumatoid SF supernatants were collected following centrifugation at 1800 rpm for 20 min and depletion of cells and particulate debris. Supernatants of SF were then aliquoted and immediately stored at −80°C. Mononuclear cells from PB (PBMC) and SF (SF mononuclear cells) were isolated using Ficoll–Hypaque density gradient centrifugation (Lymphoprep; Axis-Shield PoC). Cells were incubated with allopurinol-conjugated mouse anti-human CD3 (H1B19), CD20 (2H7) mAbs and PE-labeled CD56 (B159) mAb. CD3+ T cells and lineage-negative (Lin−) cells (CD3+, CD14+, CD16−, CD19−, CD20−, and CD56+) were then sorted using a FACS (FACSaria; BD Biosciences). The Lin− cells were seen to be a DC-enriched population (24). In some experiments, to avoid CD3-specific Ab-mediated T cell activation, we sorted CD4+ and CD8+ cells following the staining with allopurinol-conjugated mouse anti-human CD4 (RPA-T4) and anti-CD8 (RPA-T8) mAbs using FACS. The purity of sorted cells was consistently >98%, as revealed by immunofluorescence analysis.

35C-GAPDH-positive DCs (IDO+ DC) were generated from healthy donor-derived PBMC, as previously described, with some modifications (12, 15). In brief, monocytes separated from PBMC were cultured for 7 days with 25 ng/ml GM-CSF (R&D Systems) and 50 ng/ml IL-4 (R&D Systems) in serum-free X-vivo15 medium (BioWhittaker). The Lin− cells wereseen to be a DC-enriched population (24). In some experiments, to avoid CD3-specific Ab-mediated T cell activation, we sorted CD4+ and CD8+ cells following the staining with allopurinol-conjugated mouse anti-human CD4 (RPA-T4) and anti-CD8 (RPA-T8) mAbs using FACS. The purity of sorted cells was consistently >98%, as revealed by immunofluorescence analysis.

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**Quantitative PCR**

Total RNA was extracted from the sorted CD3+ T cells and DC using the guanidine thiocyanate-phenol-chloroform method modified for TRizol (Invitrogen Life Technologies). IDO transcripts were detected through RT-PCR, whereas cDNA of TTS was quantified through real-time PCR tech- nique, all with GAPDH as control. Primers for IDO were as follows: forward, 5'-CTGGGAGGCACTGATTTA-3' and reverse, 5'-ATGATCGTCTGCTTCTTTA-3'. Real-time PCR were performed using a SYBR green PCR mix and conducted with the ABI Prism 7900HT (Applied Biosystems). Thermo- cycler conditions included an initial holding at 50°C for 2 min, then 95°C for 10 min; this was followed by a two-step PCR program: 95°C for 15 s and 60°C for 60 s for 40 cycles. Data were collected and quantitatively analyzed on an ABI PRISM 7900 sequence detection system (Applied Biosystems). The primer sequences were designed using the Primer Express Software version 2.0 provided with the ABI Prism 7900HT (TTS, forward, 5'-GAGAGAGCTCTCA-TCTGTTA-3' and reverse, 5'-CTGAGGTTGAGCGA-3'). GAPDH, forward, 5'-GGGATCCGATGCA-3' and reverse, 5'-TG AGTGCGCGATAGTCA-3'). The GAPDH gene was used as an endoge- nous control to normalize for differences in the amount of total RNA in each sample. All values were expressed as fold increase or decrease relative to the expression of GAPDH. The mean value of the replicates for each sample was calculated and expressed as cycle threshold (CT) value at which each PCR reaches a predetermined fluorescence threshold (within the linear range of all reactions). The amount of gene expression was then calculated as the difference (ΔCT) between the CT value of the sample for the target gene and the mean CT value of that sample for the endogenous control (GAPDH). Rel- ative expression was calculated as the difference (ΔΔCT) between the ΔCT values of the test sample and of the control sample. Relative expression of genes of interest was calculated and expressed as 2−ΔΔCT.

**Western immunoblot analysis**

Cells were harvested, pelleted by centrifugation, and resuspended in lysis buffer. Equal amounts of protein (20 μg) were loaded onto a 5% acryl- amide stacking gel and separated by SDS-PAGE using a 10% separating gel. Following transfer of separated proteins, nitrocellulose membranes were blocked and probed overnight at 4°C with mouse anti-IDO mAb (Chemicon International). The membrane was then probed for 1 h at room temperature with goat anti-mouse peroxidase-conjugated IgG (Kirkegaard & Perry Laboratories), and the immunoreactivity was detected by chemi- luminescence. To quantify IDO proteins, each band density was normal- ized to actin protein.

**Histology and immunohistochemical staining**

Cryosections of ST from RA patients or patients with traumatic lesions were routinely stained with H&E. For IDO staining, cryosections were fixed in 10% formalin for 10 min and then treated with mouse anti-IDO mAb (Chemicon International), followed by staining of peroxidase-con- jugated rabbit anti-mouse IgG (1:200; Kirkegaard & Perry Laborato- ries), and development with 3,3′-diaminobenzidine. Sections were finally counterstained with hematoxylin and examined microscopically.

**HPLC assay**

DC were cultured in the RPMI 1640 medium supplemented with 10% FCS, 100 IU/ml penicillin, 100 μg/ml streptomycin, and 2 mM l-glutamine (complete medium). Twenty-four hours after culture, supernatant was col- lected and its kynurenine concentration was measured by HPLC. HPLC was performed, as previously described (25), with minor modification. In brief, 200 μl of supernatant was diluted with 200 μl of potassium phos- phate buffer (0.05 M; pH 7.4), and protease inhibitors (1 μM PMSF, 2 μM dithiothreitol, and 2 μM phenylmethanesulfonyl fluoride) were added. The mixture was incubated for 20 min at room temperature. 2 M trichloroacetic acid. One hundred fifty microliters of supernatants was then injected into an RP-18 column and eluted with a degassed potassium phosphate solution (0.015 M; pH 6.4) containing 27 mL/L acetonitrile at a flow rate of 0.8 ml/min. Kynurenine was detected using a UV detector at a wavelength of 365 nm. The values were referred to a standard curve with defined kynurenine concentrations (0–60 μM). To analyze cellular tryptophan use, purified T cells were cultured in complete medium with 50 μM added tryptophan at 37°C in 5% CO2. Twenty-four hours after culture, 100 μl of supernatant was collected for determination of tryptophan concentra- tion. Preparation of protein-free supernatant was the same as for kynure- nine measurement. Tryptophan concentration was measured using a fluo- rescence detector at the excitation wavelength of 285 nm and emission wavelength of 365 nm.

**Mixed leucocyte reaction**

MLR was performed by addition of DC (1 × 104 cells/well) to allogeneic or autologous T cells (1 × 105 cells/well) in RPMI 1640 medium supple- mented with 10% FCS without addition of tryptophan in 96-well U-bottom microtiter tissue culture plates (BD Biosciences). Cultures were set up in the presence or absence of IDO inhibitor, 1-methyl-1-tryptophan (1-MT, 150 μM; Sigma-Aldrich). For T cells stimulated by autologous DC, 1 μl purified anti-human CD4 (OKT3) mAb (eBioscience) was added to the medium. Triplicate wells were cultured for each group in MLR. Cells were stimulated ex vivo for 5 days, and 1 μCi/well [3H]thymidine was added 18 h before the end of culture. The cells were then harvested onto
glass fiber filters for measurement of [3H]thymidine incorporation based upon scintillation counting. In parallel with each MLR, T cells alone were cultured as controls for [3H]thymidine incorporation. In some experiments, cellular apoptosis was determined using an annexin V-FITC apoptosis detection kit I, according to the manufacturer’s instructions (BD Biosciences). In brief, after 5-day MLR, cells were harvested, stained with FITC-labeled annexin V and propidium iodide (PI) for 15 min at room temperature, and analyzed by FACS within 1 h. Necrotic cells were excluded by gating on PI-negative (PI\(^{-}\)) cells, and cell externalization of phosphatidylserine was monitored by its binding to annexin V.

**TTS induction**

PBMC from healthy donors were cultured in complete medium with IFN-γ (PeproTech) (23), or with SF supernatant at the indicated dilutions, as previously described (26), in 24-well plates at a concentration of 1 \times 10^6 cells/ml/well. Forty-eight hours after culture, cells were harvested and T cells were sorted using FACS for RNA abstraction, tryptophan concentration detection, and MLR assay. TTS expression in induced T cells was measured by real-time PCR. In some experiments, additional neutralizing mouse anti-human mAb were added to the SF-treated cultures to counter IL-18 (125-2H), IL-10 (23738), IFN-γ (25718), and/or TNF-α (6401) (5 \mu g/ml each; R&D Systems), with mouse IgG as control.

**Statistics**

Significant differences were evaluated with Student’s \(t\) tests, except that multiple treatment groups were compared within individual experiments by ANOVA or Kruskal-Wallis test. Data are expressed as the mean ± SD, and probability values of <0.05 were considered significant.

**Results**

DC from joint SF of RA patients express functional IDO

To determine the role of IDO in autoreactive T cell persistence in RA patients, we first measured the expression of IDO in DC and CD3\(^+\) T cells that were derived from RA patients, and compared it with that of cells from healthy control donors. Both RT-PCR and Western blot analysis showed that IDO was detected only in DC derived from joint SF (SF DC) and, to a lesser extent, PB (PB DC) of RA patients, and SF T cells of RA patients, or the control PB T cells of healthy donors (Fig. 1, A and B). Immunohistochemically stained for IDO. IDO staining appeared brown, and sections were counterstained with hematoxylin. Original magnification, ×100. These results are representative of five independent experiments.

**FIGURE 1. IDO expression in DC of joint synovium from RA patients.** A, IDO gene expression was analyzed through RT-PCR. RNA was prepared from FACS-sorted DC and CD3\(^+\) T cells derived from SF or PB of RA patients, with cells from PB of healthy donors as controls. Expression of IDO mRNA was normalized to GAPDH. B, PB or SF DC from RA patients and PB DC from healthy donors were subjected to Western blot analysis using mouse anti-human IDO mAb. As a normalized control, expression of actin was analyzed in parallel. C, IDO expression of ST was detected in situ by immunohistochemistry. Cryosections of ST from patients with RA were prepared and immunostained, as described in Materials and Methods. ST from patients with traumatic lesions was used as controls. Tissues were stained with H&E or immunohistochemically stained for IDO. IDO staining appeared brown, and sections were counterstained with hematoxylin.

Original magnification, \(\times 100\). These results are representative of five independent experiments.
DC of RA patients and PB DC of healthy donors induced the proliferation of allogeneic PB T cells from healthy donors. Addition of IDO inhibitor 1-MT did not affect the proliferation of allogeneic T cells in these ex vivo cultures (Fig. 3A). In contrast, whereas RA patient-derived SF DC that expressed high levels of IDO also stimulated allogeneic T cell proliferation, addition of 1-MT greatly enhanced the proliferation of these allogeneic T cells (Fig. 3A). These results suggest that SF DC of RA patients express functional IDO that is able to catabolize tryptophan, leading to the inhibition of T cell proliferation.

RA patient-derived SF T cells resist IDO-mediated suppression

We next determined the ability of SF T cells derived from RA patients to proliferate in response to IDO-positive DC. RA patient-derived SF T cells were cultured in the presence of allogeneic IDO-positive SF DC, with stimulation of PB T cells from RA patients and PB T cells from healthy donors, respectively, with the same allogeneic SF DC as controls. We found that all cultured T cells proliferated in response to allogeneic IDO-positive SF DC (i.e., 29,136 ± 3,600.2 cpm for SF T cells of RA patients; 33,990.2 ± 5,411.7 cpm for PB T cells of RA patients; and 45,385.2 ± 6,725.4 cpm for PB T cells of healthy donors; Fig. 3B). Interestingly, addition of 1-MT to the cultures significantly enhanced the proliferation of PB T cells of healthy donors (75.3%) and PB T cells of RA patients (71.6%), when compared with SF T cells of RA patients (18.7%; Fig. 3B). These results suggest that the inhibitory effect of SF DC-derived IDO on T cell proliferation is markedly ablated in SF T cells from RA patients. This conclusion was further confirmed by the culture of RA patient-derived SF T cells stimulated with autologous SF IDO-positive DC together with anti-CD3 mAb, with or without addition of 1-MT. As shown in Fig. 3C, whereas both PB T cells of healthy donors and SF T cells of RA patients proliferated to a similar extent in response to IDO-positive autologous DC plus anti-CD3 mAb, addition of 1-MT significantly enhanced the proliferation of PB T cells from healthy donors 4-fold more than that of SF T cells from RA patients. Taken together, these data suggest that, although the expression of functional IDO is increased in synovial DC and tissues of RA patients, RA patient-derived SF T cells have developed the ability to resist IDO-mediated tryptophan deprivation.

Enhanced expression of TTS in synovial T cells from RA patients

Previous studies have demonstrated that TTS induces the association of tryptophan with its specific tRNA. This tryptophan-tRNA complex enhances the storage of tryptophan and subsequent protein synthesis (22, 23). To determine whether TTS-mediated formation of tryptophan-tRNA complex accounts for the resistance of SF T cells from RA patients to IDO-mediated deprivation of tryptophan, RNA was prepared from SF T cells and PB T cells of RA patients and PB T cells of healthy donors or from patients with RA as controls. Kynurenine in the culture supernatant was measured by HPLC. Results are expressed as the mean ± SD of 10 samples in each group. *p < 0.05 significance compared between the groups.

FIGURE 2. IDO activity in DC from SF of RA patients to functionally metabolize tryptophan. SF DC derived from patients with RA were sorted by FACS, as described in Materials and Methods, and cultured in complete medium with additional tryptophan for 24 h, with PB DC from healthy donors or from patients with RA as controls. Kynurenine in the culture supernatant was measured by HPLC. Results are expressed as the mean ± SD of 10 samples in each group. *p < 0.05 significance compared between the groups.

FIGURE 3. Synovial T cells from RA patients are resistant to IDO-mediated suppression. The indicated DC (1 × 10⁵ cells/well) and T cells (1 × 10⁵ cells/well) were incubated in 200 µl of complete medium per well in 96-well U-bottom microtest tissue culture plates in the presence or absence of 150 µM 1-MT, an inhibitor of IDO. T cell proliferation was measured by incorporation of [³H]thymidine added during the last 18 h of the 5-day incubation. A, SF DC and PB DC derived from RA patients were used as stimulators and were cultured with allogeneic PB T cells from healthy donors, with PB DC from healthy donors as controls. B, SF T cells derived from RA patients as responders were stimulated with allogeneic SF DC, with PB T cells from RA patients or from healthy donors as controls. C, RA-SF T cells were stimulated with autologous SF DC in the presence of 1 µg/ml anti-CD3 mAb; PB T cells from healthy donors were stimulated in parallel with autologous IDO⁺ DC, which were ex vivo induced from PBMC-derived monocytes, as described in Materials and Methods. Inset, Representative histogram of IDO expression of the ex vivo induced DC. Results are expressed as the mean ± SD of triplicate cultures of 10 samples in each group. □, Represent cultures without 1-MT; ■, with 1-MT. *p < 0.05 significance compared between the groups.
patients, with PB T cells of healthy donors used as controls. Real-time PCR showed that SF T cells of RA patients expressed significantly more TTS transcripts than PB T cells from healthy donors or from RA patients (n = 12), with a panel of PB T cells of healthy donors as control. TTS expression was normalized to endogenous GAPDH in the same samples and expressed as 2^{ΔΔCt} as instructed in Materials and Methods. B, Tryptophan capacity of T cells from SF, paired PB of RA patients, or PB of healthy donors was measured through detection of tryptophan in their culture supernatant. T cells were cultured in complete medium with 50 μM added tryptophan for 24 h, and levels of tryptophan concentration were analyzed using HPLC. Results are expressed as the mean ± SD of triplicate cultures of eight samples in each group. In all cases, asterisks indicate statistically significant differences between the groups (p < 0.05).

**FIGURE 4.** Analysis of TTS expression and tryptophan consumption in T cells from SF of RA patients. A, Real-time PCR analysis of TTS expression in T cells isolated from SF and paired PB of RA patients (n = 12), with a panel of PB T cells of healthy donors as control. TTS expression was normalized to endogenous GAPDH in the same samples and expressed as 2^{ΔΔCt} as instructed in Materials and Methods. B, Tryptophan capacity of T cells from SF, paired PB of RA patients, or PB of healthy donors was measured through detection of tryptophan in their culture supernatant. T cells were cultured in complete medium with 50 μM added tryptophan for 24 h, and levels of tryptophan concentration were analyzed using HPLC. Results are expressed as the mean ± SD of triplicate cultures of eight samples in each group. In all cases, asterisks indicate statistically significant differences between the groups (p < 0.05).

**FIGURE 5.** Tryptophan consumption of T cells was associated with their levels of TTS expression. A, TTS expression in IFN-γ-induced T cells. PBMC from healthy donors were incubated in complete medium with or without IFN-γ for 48 h; T cells were then isolated by FACS. TTS mRNA expression of the isolated T cells was calculated through real-time PCR by comparing with GAPDH and was expressed as 2^{ΔΔCt}. B, Tryptophan concentration of the culture supernatant of IFN-γ-induced T cells was determined by HPLC. IFN-γ-treated T cells expressing increased levels of TTS were termed TTS^{high} T cells, whereas those left IFN-γ untreated were termed TTS^{low} T cells. Cells were cultured for 24 h in complete medium with 50 μM added tryptophan. In parallel, tryptamine, a TTS inhibitor, was added to the TTS^{high} T cell cultures to suppress the function of TTS. C, Correlation curve between tryptophan consumption and level of TTS expression. Data are taken from experiments on the in vitro induced TTS^{high} and TTS^{low} T cells, and TTS expression is expressed as –ΔΔCt; r² = 0.9686. Results are presented as the mean ± SD of 21 samples. In all cases, asterisks indicate statistically significant differences between the groups (p < 0.05).
cultures markedly enhanced the proliferation of TTSlow, but not TTShigh T cells (Fig. 6A). Supernatants from the culture of TTShigh T cells stimulated with IDO-positive DC contained a markedly reduced amount of tryptophan catabolite kynurenine (5.7 \( \pm \) 0.9262 M) as compared with that of TTSlow T cells (14.9 \( \pm \) 2.1826 M). Flow cytometric analysis further revealed that more T cells underwent apoptosis in the TTSlow group stimulated by IDO-positive DC than in the TTShigh T cell group (44.1 vs 10.0%; Fig. 6B). The findings indicated that enhanced expression of TTS in T cells was responsible for their resistance to IDO-mediated tryptophan deprivation and apoptotic cell death.

RA patient-derived SF enhances TTS expression in T cells

Finally, we tested whether factors in SF of RA patients play an important role in up-regulating the expression of TTS in SF T cells. As shown in Fig. 7A, stimulation of PBMC from RA patients or healthy donors with RA patient-derived SF, but not with sera from the same patient, increased the expression of TTS in T cells as many as 9-fold as compared with unstimulated T cells. This SF-mediated up-regulation of TTS expression was SF dose dependent (Fig. 7B). Addition of neutralizing mAb to IFN-\( \gamma \) or to TNF-\( \alpha \), but not to IL-18 and IL-10, blocked the elevation of TTS in healthy donor-derived T cells stimulated by RA patient-derived SF (Fig. 7C). Thus, proinflammatory cytokines in the SF of RA patients, including IFN-\( \gamma \) and TNF-\( \alpha \), play pivotal roles in up-regulating TTS in T cells.

Discussion

Catabolism of tryptophan by IDO plays an important role in regulating T cell-mediated immune responses and inflammation (27, 28). We demonstrated that whereas DC freshly isolated from PB of both healthy donors and RA patients did not express detectable IDO protein, SF DC from RA patients expressed high levels of functionally active IDO. Although IDO produced by SF DC inhibited the proliferation of PB T cells derived either from healthy
donors or RA patients, synovial T cells from RA patients resisted the suppressive effect of IDO. We found that these synovial T cells from RA patients expressed high levels of TTS and were able to preserve tryptophan, which appears to be responsible for their resistance to IDO-mediated tryptophan deprivation. Furthermore, blocking proinflammatory cytokines, including TNF-α and IFN-γ, abrogated the effect of RA patient-derived SF on up-regulating TTS expression in T cells. Compared with TTS<sub>low</sub> T cells, T cells with increased expression of TTS gained the ability to resist IDO-mediated tryptophan deprivation. Thus, it is likely that proinflammatory cytokines in SF of RA patients play important roles in up-regulating TTS expression in T cells, leading to the resistance of T cells to tryptophan deprivation by IDO and the subsequent persistence of inflammatory synovial T cells in RA patients.

Tryptophan is an essential amino acid that is required by all cells to synthesize proteins. The depletion of tryptophan and the accumulation of proapoptotic tryptophan-derived catabolite may arrest proliferating cells in mid-G1 phase and block effector activity of T cells (29). Data from previous studies indicate that IDO<sup>+</sup> DC is involved in the development of tolerance (30–35). IDO<sup>+</sup> DC suppressed Ag-specific T cell responses in vitro (36). Induction of IDO in DC by CTLA-4-Ig resulted in blockade of clonal T cell expansion (37). Furthermore, in vivo administration of 1-MT to mice, the specific inhibitor of IDO, accelerated the diseases of experimental autoimmune encephalomyelitis (38) and T cell-mediated colitis (39), in which autoreactive T cells persist during disease progression. Autoreactive T cells capable of recognizing tissue-specific Ags are not necessarily deleted in the thymus as they can be cloned from lymph nodes of mice and from the circulation of humans (40). However, the manner in which these self-reactive T cells are exquisitely regulated remains controversial. Data from recent studies indicate that regulatory DC expressing high levels of IDO play important roles in modulating autoimmune responses (27). In our studies, we found that although SF DC from patients with RA expressed functional IDO, synovial T cells isolated from these patients resisted IDO-mediated suppression. These observations suggest that the resistance of synovial T cells to IDO-mediated tryptophan deprivation is an important mechanism responsible for the persistence of inflammatory T cells in the joints of RA patients.

The resistance of synovial T cells to IDO-mediated deprivation of tryptophan is associated with the increased expression of TTS. Data from other investigators suggest that TTS protects T cells from IDO-mediated cell injury by inducing the formation of tryptophan-tRNA complex. This outcome benefits from the lower Michaelis constant (Km) of TTS for tryptophan than that of IDO (18, 41–43). This may result not only in significantly increased preservation of intracellular tryptophan-tRNA complex for protein synthesis, but also in the reduction of cytotoxic tryptophan catabolites mediated by IDO. Indeed, our findings indicate that stimulation of TTS<sub>high</sub> T cells with IDO-positive DC leads to more markedly decreased tryptophan catabolites kynurenines (5.7 μM) than does stimulation of TTS<sub>low</sub> T cells (14.9 μM). Such stimulation was accompanied by substantially reduced apoptosis in TTS<sub>high</sub> T cells as compared with that of TTS<sub>low</sub> T cells. This supports the observations from previous studies that the tryptophan catabolites are cytotoxic to T cells (44). It is likely that by use of tryptophan, TTS has dual protective effects on T cells toward IDO-mediated cell suppression, which increases survival of inflammatory T cells in inflamed rheumatoid synovium.

The exact causal relationship between IDO and TTS is not entirely known. Previous studies have demonstrated that whereas IDO is mainly expressed in macrophages, DC, and placental trophoblasts (22), TTS is constitutively expressed in various types of cells, but can be markedly up-regulated in cells such as T cells, fibroblasts, and monocytes (21, 45–48). Interestingly, both IDO and TTS can be induced by IFN-γ (21, 22). It is conceivable that cells expressing an increased level of TTS are protected in an environment in which increased IDO leads to cell suppression. Indeed, there is recent evidence indicating that TTS protects both DC and CD8<sup>+</sup> T cells against IDO-mediated tryptophan deprivation and cytotoxic catabolites of tryptophan (23). Similar protective effects on host cells expressing high levels of TTS against IDO-mediated cell suppression have also been reported in host cells infected by pathogens (49). In our study, we showed that whereas synovial DC expressed high levels of active IDO, synovial, but not peripheral T cells from RA patients had substantially higher TTS expression. It appears that the protective mechanism of TTS toward the suppressive effect of IDO plays an important role in survival of pathogenic T cells in inflamed rheumatoid synovium.

It is intriguing to investigate how TTS is regulated in RA-SF T cells. Data from prior studies have demonstrated that IFN-γ can enhance the expression of TTS in T cells (46, 50). We found that RA patient-derived SF contained high levels of inflammatory cytokines IFN-γ and TNF-α that are responsible for up-regulating the expression of TTS in T cells. Although SF supernatant derived from RA patients increased expression of TTS in T cells from healthy donors, addition of neutralizing mAbs to IFN-γ and or TNF-α, but not mAb to IL-18 or IL-10, markedly suppressed the expression of TTS in SF-treated T cells. Furthermore, because TTS was up-regulated in normal T cells in response to RA patient-derived SF, we suggest that the synovial environment of RA patients may be another major target for potential therapeutic intervention toward synovial inflammatory T cells in RA.

It is also of interest to understand that IFN-γ has dual functions in immune response. In rheumatoid synovium, although IFN-γ increases IDO in DC, it can also induce T cells to express high levels of TTS. Previous studies have demonstrated that IFN-γ is able to increase the expression of TTS in various types of cells as early as 6 h after exposure to IFN-γ (21, 51). Notably, treatment with IFN-γ for 18 h led to a 4-fold increase in the expression of TTS in human fibroblasts as opposed to that of control cells (45). These findings collectively suggest that TTS induction by IFN-γ can be mediated independently from de novo protein synthesis (21). Our results described in this study confirm that human T cells, upon treatment with IFN-γ, substantially up-regulate the expression of TTS within 24–48 h, which is in agreement with previous reports (21, 45). Thus, although IFN-γ can also increase IDO in DC, the superior ability of TTS to IDO in competing for tryptophan in IFN-γ-activated T cells (18, 41–43) results in an augmented intracellular reservoir of tryptophan for protein synthesis and in decreased cytotoxic catabolites mediated by IDO. Further investigation of molecular mechanisms by which IFN-γ increases the expression of TTS in inflammatory cells will be important for understanding the pathological role of synovial inflammatory T cells in RA.

In summary, our data provide evidence for a link between impaired T cell response toward tryptophan depletion and environmental autoreactivity in RA patients. We demonstrated that increased TTS expression in T cells allows resistance to IDO-mediated tryptophan deprivation, which may contribute to the persistence of autoreactive T cells in local tissues of patients with autoimmune disease. Given the importance of tryptophan metabolism in regulating T cell-mediated immune response, our findings provide new insight into the pathophysiology of synovial inflammation in RA patients and development of novel therapeutic approaches for RA.
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Disclosures

The authors have no financial conflict of interest.

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