Endocr (2009) 36:119–125
DOI 10.1007/s12020-009-9184-0

REVIEW

Increased TTS abrogates IDO-mediated CD4$^+$ T cells suppression in patients with Graves' disease

Shu Wang · Chaoming Mao · Zefei Zhao · Qiaoli Gu · Min Jin · Yichuan Xiao · Xiaohua Jiang · Yongju Zhao · Yanyun Zhang · Guang Ning

Received: 4 February 2009 / Accepted: 24 March 2009 / Published online: 11 April 2009

Abstract Indoleamine 2,3-dioxygenase (IDO)-expression in antigen-presenting cells (APCs) may control autoimmune responses by depleting the available tryptophan, whereas tryptophanyl-tRNA synthetase (TTS) may counteract this effect. The study aims to determine whether abnormal IDO and TTS activities in autoreactive T, B and dendritic cells (DCs) lead to tryptophan metabolism disorder, inducing the immune imbalance in patients with Graves' disease (GD). The concentrations of serum kynurenine and tryptophan and the mRNA expressions of IDO and TTS were analyzed, and the mixed leukocyte reaction (MLR) was employed to assess the interaction of IDO-expressing DCs and TTS-expressing CD4$^+$ T cells. Compared with healthy donors (HD), the ratio of serum kynurenine to tryptophan ($P < 0.0001$) was increased in GD patients, which was associated with the increased IDO expression in B cells ($P < 0.01$) and DCs ($P < 0.01$). GD-derived CD4$^+$ T cells enhanced TTS expression ($P < 0.01$), and its proliferation was not inhibited in the presence of IDO-expressing DCs from the GD patients. In contrast, the proliferation of HD-derived CD4$^+$ T cells with low TTS expression was inhibited. Increased TTS expression from CD4$^+$ T cells resists IDO-mediated immunosuppression from DCs, which might link to a pathogenic mechanism involved in autoreactive T cells being sustained in vivo in GD patients.

Keywords Indoleamine 2,3-dioxygenase · Tryptophanyl-tRNA synthetase · Graves' disease · Interferon-γ

Shu Wang and Chaoming Mao contributed equally to this study.

S. Wang · Z. Zhao · X. Jiang · Y. Zhao · G. Ning (✉)
Department of Endocrine, Ruijin Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, 197 Ruijin 2nd Road, Shanghai 200025, China
e-mail: guangning@medmail.com.cn

S. Wang · Z. Zhao · X. Jiang · Y. Zhao · G. Ning
Laboratory of Endocrinology and Metabolism, Institute of Health Sciences, Chinese Academy of Sciences, 225 South Chongqing Road, Shanghai 200025, China

C. Mao · Q. Gu · M. Jin · Y. Xiao · Y. Zhang (✉)
Shanghai Institute of Immunology, Institutes of Medical Sciences, Shanghai Jiao Tong University School of Medicine and Institute of Health Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, 227 South Chongqing Road, Shanghai 200025, China
e-mail: yyzhang@sibs.ac.cn

Introduction

Graves' disease (GD) is a common organ-specific autoimmune disease. Previous studies have demonstrated antibody against the thyrotropic-stimulating hormone (TSH) receptor (TRAb), an agonistic autoantibody, mimics the stimulatory effects of TSH, causing hyperthyroidism and diffuse hyperplasia of the thyroid gland [1–3]. However, the mechanisms in the breakdown of the immune tolerance to TSH receptor that leads to TRAb production remain obscure.

Tryptophan is an essential amino acid for cell survival and proliferation [4]. Indoleamine 2,3-dioxygenase (IDO), a first and rate-limiting enzyme, can convert tryptophan into kynurenine, which in turn inhibits the proliferation of various cells including the immune cells. It is demonstrated that the activation of IDO has much bearing on the failure of immune surveillance on tumor cells [5, 6], and the development of autoimmune diseases such as multiple sclerosis, autoimmune diabetes, and rheumatoid arthritis

[7–9]. In contrast, tryptophanyl-tRNA-synthetase (TTS) is a constitutively expressed cytoplasmic enzyme with the ability to mediate tryptophan to bind with its specific tRNA. The tryptophan and its tRNA complex form a reservoir of tryptophan for protein synthesis, which can antagonize IDO-mediated immunosuppression by depleting the available tryptophan [10–12]. Since IDO is mainly expressed in antigen-presenting cells (APCs), such as macrophages and dendritic cells (DCs), the activation of IDO in APCs may cause a local decrease in tryptophan concentration, suppressing the activation of the surrounding T lymphocytes [4], whereas TTS just works in the opposite way.

IDO and TTS are involved in the regulation of immune response via modulating the tryptophan concentration. The aim of our study is to determine whether abnormal IDO and TTS activities in autoreactive T, B cells and DCs lead to the tryptophan metabolism disorder, inducing immune imbalance in GD patients. In this study, we found the ratio of serum kynurenine to tryptophan (Kyn/Trp) was increased, which was associated with the increased IDO expression in B cells and DCs. However, increased TTS expression in $CD4^+$ T cells induced by the inflammatory cytokine IFN-γ crippled IDO-mediated immunosuppression, contributing to the development and progression of GD. These results give us thought that the efficacy of the therapy for GD patients might be improved by inhibiting TTS expression.

Materials and methods

Subjects

Fifty patients were recruited in this study from the Outpatient Department of Ruijin Hospital Affiliated to Shanghai Jiaotong University School of Medicine. All patients, without previous treatment, were newly diagnosed by experienced endocrinologists through standard clinical and laboratory examination. GD patients were identified by clinically and biochemically verified hyperthyroidism and positive TRAb. The clinical evaluation included patients' history, physical examination, and thyroid ultrasound. The laboratory examinations included free triiodothyronine (FT3), free thyroxine (FT4), sensitive thyroid-stimulating hormone (s-TSH), antibodies against thyroperoxidase (TPOAb), thyroglobulin (TGAb), and TRAb. The following ranges were considered normal: FT3: 2.62–6.48 pmol/l, FT4: 9.01–19.04 pmol/l, s-TSH: 0.35–4.94 mIU/l, TgAb: <0.30, TPOAb: 2.62–6.48 pmol/l; TRAb: <1.5 IU/l. Thirty sex- and age-matched healthy donors (HD) as controls were selected based on the normal outcome of physical examination, endocrine function, and levels for TGAb, TPOAb and TRAb. The lab examination results of the patients and HD are summarized in Table 1. Informed consent was obtained from all participants before the samples were collected. The study protocol was approved by the Ethics Committee of Shanghai Jiaotong University School of Medicine.

Table 1 Clinical data of GD patients and HD

	GD	HD
n	50	30
Female *n* (%)	41(82%)	24(80%)
Age (year)	45 ± 13	41 ± 11
FT3 (pmol/l)	17.40 ± 6.06	3.61 ± 1.24
FT4 (pmol/l)	40.70 ± 11.41	14.98 ± 3.76
s-TSH (mIU/l)	0.011 ± 0.004	2.04 ± 1.25
TPOAb (pmol/l)	82.32 ± 49.55	3.78 ± 2.54
TGAb	0.27 ± 0.12	0.06 ± 0.04
TRAb (IU/l)	24.15 ± 10.83	0.59 ± 0.37

Values are expressed as mean ± SD

GD Graves' disease, *HD* healthy donor, *FT3* free triiodothyronine, *FT4* free thyroxin, *s-TSH* sensitive thyroid-stimulating hormone, *TGAb* antibody against thyroglobulin, *TPOAb* antibody against thyroperoxidase, *TRAb* antibody against TSH receptor

Cell isolation and purification

According to manufacturer's instruction, human peripheral blood mononuclear cells (PBMCs) were first isolated from freshly obtained blood by Ficoll density gradient (Sigma-Aldrich). DCs with purity >90% from peripheral blood were then obtained according to the human blood DCs isolation kit II manufacturer's instruction (Miltenyi Biotec) with negative selection and positive selection methods by magnetic-activated cell sorting (MACS). Next, the residual cells were used to further isolate $CD4^+$ T cells using the $CD4^+$ T cell isolation kit (Miltenyi Biotec) according to the manufacturer's instruction. The purity of $CD4^+$ T cells were >95%. In some experiments, $CD19^+$ B cells were also isolated by the $CD19^+$ B cell isolation kit (Miltenyi Biotec) according to the manufacturer's instruction.

TTS induction in $CD4^+$ T cells

PBMCs of 1×10^6/ml from HD were cultured for 48 h in RPMI 1640 medium with 10% FBS in the absence or presence of 4 μg/ml rhIFN-γ (PeproTech). Cells were harvested at the end of 48 h and $CD4^+$ T cells were sorted out using the $CD4^+$ T cell isolation kit. TTS expression in induced T cells was measured by real-time RT-PCR. Additional neutralizing rhIFN-γ mAb (R&D Systems) was added to the cultures in some experiments.

Real-time quantitative RT-PCR

Total RNA was extracted from PBMCs, isolated $CD4^+$ T cells, $CD19^+$ B cells or DCs using RNeasy Mini Kit (Qiagen) and transformed into cDNA with reverse transcription kit (Promega). cDNA was amplified with primers specific for IDO (5′-ACTGGAGGCACTGATTTA-3′ and 5′-ATTAGTTTGTGGCTCTGTTA-3′), TTS (5′-GAA AGGCATTTTCGGCTTCA-3′ and 5′-CAGCCTGGATGG CAGGAA-3′), and GAPDH (5′-GTGAAGGTCGGAGTC AACG-3′ and 5′-TGAGGTCAATGAAGGGGTC-3′). Real-time PCR was performed using a SYBR Green PCR Master Mix in an ABI Prism 7900HT (Applied Biosystems) under the following cycling conditions: denaturation at 94°C for 10 min, followed by 40 cycles at 95°C for 30 s, 55°C for 60 s, and 72°C for 60 s. The GAPDH gene was used as an endogenous control to normalize 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*; cycle number at which each PCR reaches a predetermined fluorescence threshold, set 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). Relative expression was calculated as the difference (ΔCT) between the ΔCT values of the test sample and of the control sample. Relative expression of genes of interest was calculated and expressed as $2^{-\Delta\Delta}CT$.

Measurement of tryptophan and kynurenine concentrations by HPLC

Serum samples from the GD patients and HD were collected and stored in aliquots frozen at −80°C until further use. Kynurenine and tryptophan concentrations from serum were measured with high-pressure liquid chromatography (HPLC), which was performed as previously described but with minor modification [9, 13, 14]. Briefly, 400 µl of serum was diluted with 400 µl of potassium phosphate buffer (0.05 M, pH 6.0), and protein was precipitated with 100 µl of 2 M trichloroacetic acid. Supernatant of 300 µl was then injected into an RP18 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 by a UV detector at a wavelength of 360 nm. Tryptophan concentration was measured using a fluorescence detector at the excitation wavelength of 285 nm and emission wavelength of 365 nm. The values were referred to a standard curve with defined kynurenine and tryptophan concentrations (0–60 µM).

Mixed leukocyte reaction

Mixed leukocyte reaction (MLR) was performed by adding irradiated DCs (3×10^4 cells/well) to allogeneic or autologous T cells (1×10^5 cells/well) in RPMI 1640 medium supplemented with 20% GD serum and without adding tryptophan in the 96-well U-bottom plates. Cultures were set up in the presence or absence of IDO inhibitor, 1-methyl-D-tryptophan (1-MT, 150 µM; Sigma-Aldrich). To stimulate T cells by autologous DC, 0.5 µg/ml purified anti-human CD3 (OKT3) mAb (eBioscience) was added to the medium. Triplicate wells were cultured for each group in MLR. Cells were co-cultured in vitro for 4 days, and 1 µCi/well [^{3}H]TdR was added 16 h before the end of culturing. The cells were then harvested and the radioactivity was detected by the liquid scintillation counter.

Analysis of serum IFN-γ by ELISA

Serum interferon-γ (IFN-γ) was measured in the supernatant using the commercial IFN-γ ELISA kit (R&D Systems) according to the manufacturer's instruction.

Statistical analysis

Results were expressed as mean ± standard deviation (SD). Statistical comparison was performed using the Student's *t* test. Values of $P < 0.05$ were considered to be statistically significant.

Results

The ratio of serum Kyn/Try is increased in GD patients

To determine the role of IDO and TTS in GD patients, we first measured the serum concentrations of kynurenine and tryptophan from the GD patients ($n = 50$) and HD ($n = 30$), which reflect the activity of IDO and TTS. As shown in Fig. 1a, we found that serum kynurenine concentration in the GD patients was significantly higher than that in the HD (2.01 ± 0.66 vs. 1.09 ± 0.27 µM; $P < 0.0001$). In contrast, the serum tryptophan concentration in the GD patients was almost equal to those in the HD (72.27 ± 19.27 vs. 72.17 ± 19.85 µM; $P = 0.958$) (Fig. 1b). However, the kynurenine to tryptophan ratio (Kyn/Trp), an estimate of IDO activity, was significantly higher in the GD patients than that in the HD (0.028 ± 0.008 vs. 0.016 ± 0.007; $P < 0.0001$) (Fig. 1c).

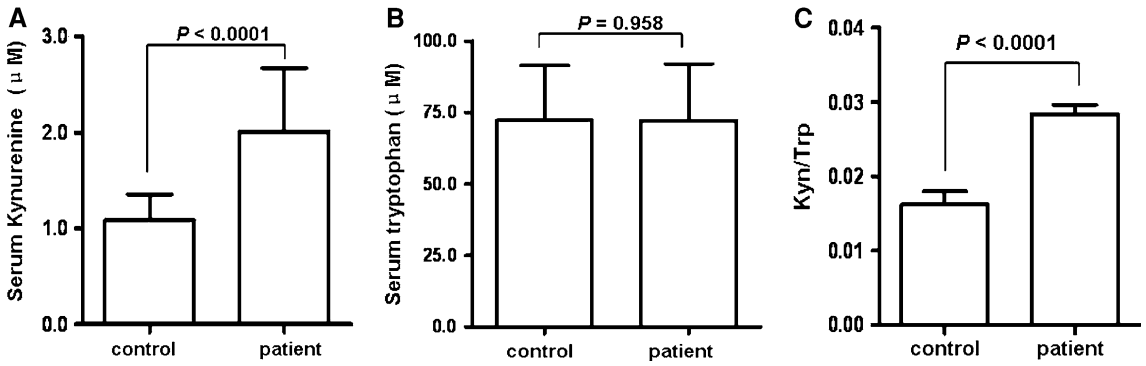

Fig. 1 The kynurenine and tryptophan concentrations in the GD patients. Serum concentrations of kynurenine (**a**) and tryptophan (**b**) from the GD patients ($n = 50$) and HD ($n = 30$) were measured by HPLC. The ratio of Kyn/Trp (**c**) was calculated. $P < 0.05$ significance compared the two groups. Results are expressed as mean ± SD

Both IDO and TTS expressions increase in GD patients

It is known that IDO and TTS jointly participate in tryptophan metabolism. We tested whether IDO and TTS expressions corresponded to the increased levels of serum kynurenine and constant serum tryptophan in the GD patients. First, IDO and TTS mRNA expressions in PBMCs from 25 of 50 GD patients were measured. Compared with those in the HD ($n = 15$), IDO mRNA expression showed a small but insignificant increase in the GD patients (1.52 ± 0.36 fold; $P = 0.12$) (Fig. 2a), whereas TTS mRNA expression had a marked increase (2.57 ± 0.98 fold; $P < 0.01$) (Fig. 2b).

To better investigate the distribution of IDO and TTS in cell subpopulations of PBMCs, we sorted out B cells, DCs, and $CD4^+$ T cells from the GD patients ($n = 25$) and HD ($n = 15$) and detected the expression of IDO and TTS in them, respectively. Real-time RT-PCR showed that IDO expression in both B cells (2.52 ± 0.65 fold; $P < 0.01$) and DCs (3.40 ± 0.79 fold; $P < 0.01$) of the GD patients

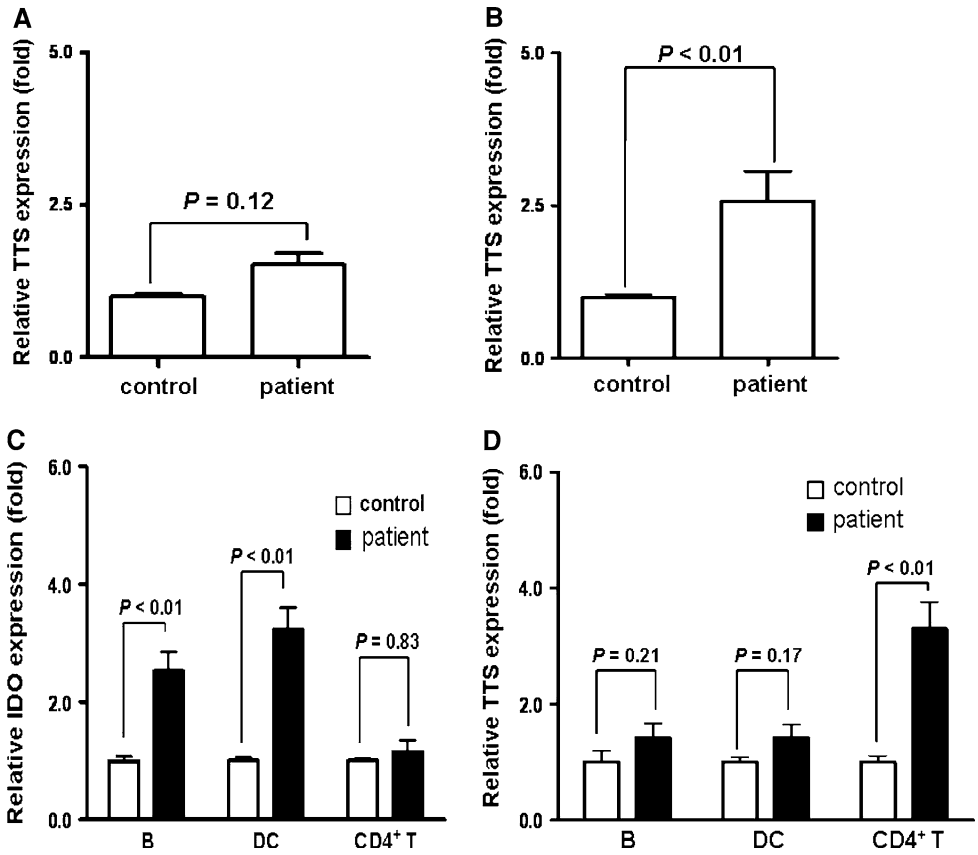

Fig. 2 The distribution of IDO and TTS mRNA expression in the GD patients. Total RNA was isolated and reversely transcribed into cDNA. The expressions of IDO and TTS mRNA were analyzed by real-time RT-PCR. IDO (**a**) and TTS (**b**) expressions in PBMCs from the GD patients ($n = 25$) and HD ($n = 15$) were shown. B cells, DCs, and $CD4^+$ T cells were isolated by MACS, IDO (**c**), and TTS (**d**) expression in B cells, DCs and $CD4^+$ T cells from the GD patients ($n = 25$) and HD ($n = 15$) were shown, respectively. $P < 0.05$ significance compared the two groups. Results are expressed as mean ± SD

significantly higher than those in HD, but not in $CD4^+$ T cells (1.35 ± 0.31 fold; $P = 0.83$) (Fig. 2c). Conversely, TTS expression in $CD4^+$ T cells (3.47 ± 0.77 fold; $P < 0.01$) of the GD patients were significantly higher than that in HD, but no difference either in B cells (1.56 ± 0.64 fold; $P = 0.21$) or in DCs (1.67 ± 0.74 fold; $P = 0.17$) (Fig. 2d).

Increased IFN-γ induces TTS expression in T cells

To determine whether the changes of IDO and TTS expressions in the GD patients were associated with IFN-γ secretion, the level of serum IFN-γ in the GD patients was measured. As shown in Fig. 3a, serum IFN-γ in the GD patients ($n = 50$) was significantly increased compared to the HD ($n = 30$) (22.76 ± 10.83 vs. 13.10 ± 7.22 ng/ml; $P < 0.01$).

Previous studies have suggested that IFN-γ is able to induce the expression of IDO in APCs [12, 15], thus elevated IFN-γ offered an explanation for increased IDO in B cells and DCs of the GD patients. To account for the relationship of increased IFN-γ with increased TTS expression in $CD4^+$ T cells of the GD patients, PBMCs from the HD were treated with IFN-γ for 48 h and TTS expression was evaluated. Real-time RT-PCR showed significant increased expression of TTS in isolated $CD4^+$ T cells from IFN-γ-treated PBMCs compared with untreated or mAb-treated $CD4^+$ T cells (7.56 ± 3.06 fold; $P < 0.01$) (Fig. 3b), demonstrating that IFN-γ was able to modulate $CD4^+$ T cells in the TTS expression up-regulation.

GD-derived $CD4^+$ T cells resist GD-derived DC-mediated immunosuppression

The orientation of immune response depends on the interaction of APCs and effector T cells. To determine the functional character of IDO-expressing DCs and TTS-expressing $CD4^+$ T cells after their interaction in the GD patients, MLR for GD-derived DCs and $CD4^+$ T cells was performed with HD-derived DCs and $CD4^+$ T cells as controls. As shown in Fig. 4, we found that GD-derived DCs had an ability to inhibit the proliferation of HD-derived $CD4^+$ T cells, and the effect was blocked by IDO inhibitor 1-MT ($P < 0.01$), indicating that GD-derived DCs with its increased IDO had an immunosuppressive effect. Of note, however, when GD-derived DCs was co-cultured with GD-derived $CD4^+$ T cells, the proliferation of $CD4^+$ T cells was not so obviously suppressed with or without 1-MT ($P = 0.84$), indicating that GD-derived $CD4^+$ T cells resist the immunosuppression from GD-derived DCs.

Discussion

IDO up-regulation in APCs by IFN-γ results in the inhibition of T-cell activation and proliferation under tryptophan deprivation from the microenvironment [13, 16–18], an important form of maintaining immune balance in the physiological condition. In general, the insufficiency of immunoregulatory ability plays an important role in the development of autoimmune disease. In our study, the

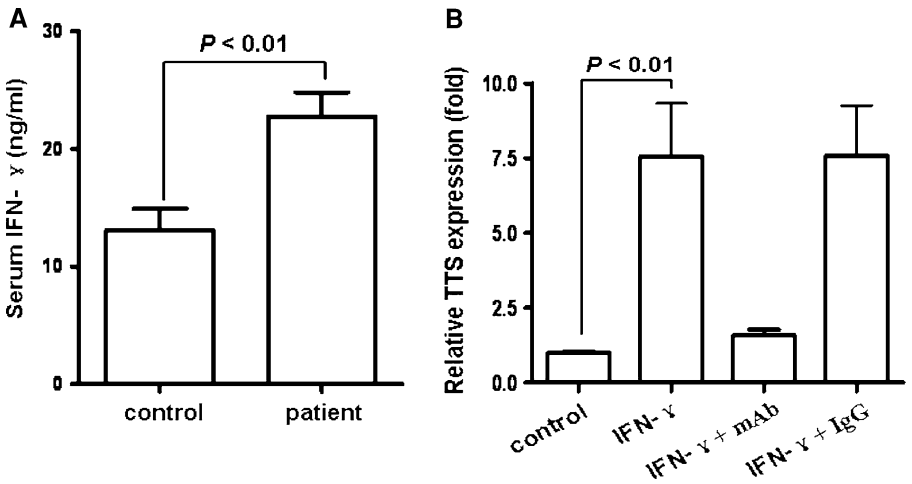

Fig. 3 Increased TTS expression in $CD4^+$ T cells from the GD patients is dependent on IFN-γ. The level of serum IFN-γ (**a**) in the GD patients ($n = 50$) and HD ($n = 30$) was measured by ELISA. $CD4^+$ T cells from the HD were isolated and incubated in complete medium with or without IFN-γ, and neutralizing test for anti-human mAb against IFN-γ was performed simultaneously. Cells were collected after cell culture for 48 h to analyze TTS expression through real-time RT-PCR. One representative of three independent experiments (**b**) was shown. $P < 0.05$ significance compared the two groups. Results are expressed as mean ± SD

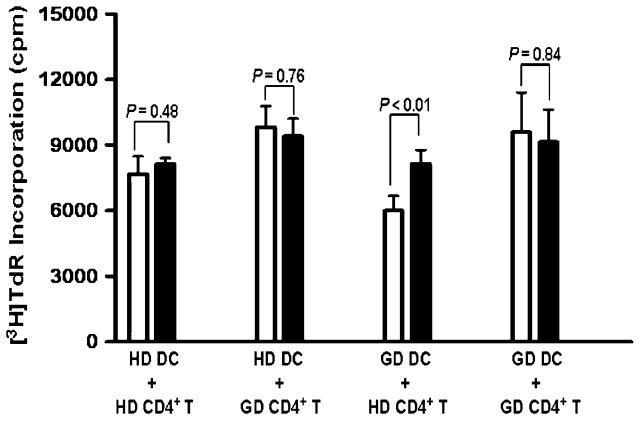

Fig. 4 $CD4^+$ T cells from the GD patients are resistant to IDO-mediated suppression. Irradiated DCs (3×10^4 cells/well) to allogeneic or autologous T cells (1×10^5 cells/well) from the HD and GD patients were incubated by mixed leukocyte reaction, respectively, as described in "Materials and methods" section. □, Cultures without 1-MT; ■, with 1-MT. $P < 0.05$ significance compared the two groups. Results are expressed as mean ± SD of triplicate wells for [^{3}H]TdR incorporation. The data are representative of three independent experiments

increase of the serum concentration of kynurenine meant amplified tryptophan depletion in the GD patients; however, the serum concentration of tryptophan did not decrease. Further results showed that GD patients had significant increase of IDO expressions in B cells and DCs. Moreover, the expression of IDO in an enzymatically active form was also confirmed by the elevated Kyn/Trp ratio. Taken together, these findings indicate that IDO, the immune inhibitor, was actually not decreased in the GD patients.

As a potent immunosuppressive agent, IDO has been widely investigated for the induction of immunologic tolerance such as inhibiting the production of antibody [19]. The elevated baseline IDO level should exert a negative effect on immune system for controlling autoimmune occurrence, for instance, autoantibody TRAb production in GD patients. However, unexpectedly, the negative capacity of IDO seems to be insufficient in the GD patients. As the roles between IDO and TTS are functionally antagonized and both involve in tryptophan metabolism, TTS could probably have an effect to prevent IDO-mediated immune suppression [12]. In this study, we found TTS expression in $CD4^+$ T cells, but not in B cells or DCs, was significantly increased in the GD patients. Previous data demonstrated that IDO is mainly expressed in APCs, which can be induced by cytokine IFN-γ [12, 15]. In our study, increased serum concentration of IFN-γ may account for the cause of the increased IDO expression in APCs of the GD patients. At the meantime, whether up-regulated TTS expression in $CD4^+$ T cells of the GD patients also ascribes to the increased serum IFN-γ is worth to be investigated. We confirmed that IFN-γ indeed significantly increased the expression of TTS in $CD4^+$ T cells by induction experiment in vitro, and the addition of neutralizing mAbs to IFN-γ markedly suppressed the expression of TTS in IFN-γ-treated T cells, showing that IFN-γ is also responsible for increased TTS expression in $CD4^+$ T cells of the GD patients. To sum up, IFN-γ in the GD patients, not only increases IDO expression in APCs, but also induces high levels of TTS expression in $CD4^+$ T cells.

It is well understood that activated T lymphocytes have a higher rate of protein synthesis compared with DCs, which should result in a higher sensitivity to IDO-induced tryptophan depletion. However, the development of GD indicates a weakness in the IDO-mediated immunosuppression. By MLR, we found that the expansion of low TTS-expressing $CD4^+$ T cells from the HD were inhibited by IDO-expressing DCs from the GD patients, but not of high TTS-expressing $CD4^+$ T cells from the GD patients, showing GD-derived $CD4^+$ T cells for TTS high expression were not susceptible to IDO-mediated immunosuppression. In other words, GD-derived $CD4^+$ T cells are able to resist the immunosuppression from self-derived DCs. Accordingly, we presume the superior ability of TTS over IDO in competing for tryptophan in IFN-γ-activated T cells [20, 21], thus maintains intracellular reservoir of tryptophan in a necessary level for protein synthesis of autoreactive $CD4^+$ T cells in the GD patients, contributing to the production of autoantibody and the development of GD.

Many studies have demonstrated that IDO mainly express in DCs [13, 15] and tumor cells [22, 23]. The former mediates immune tolerance and the latter induces immune escape. It is an intriguing issue why IDO-expressing cells themselves are not susceptible to tryptophan depletion. One possibility is that IDO-expressing cells are protected against tryptophan starvation, for instance, by increasing the expression of TTS, avoiding the negative effects of tryptophan depletion. The other possibility is that protein synthesis still proceeds despite the lowered levels of tryptophan, presumably because the Michaelis constant (Km) of TTS for tryptophan is lower than that of IDO, maintaining the growth of IDO-expressing cells themselves [22]. In general, activated T lymphocytes have a higher rate of protein synthesis compared with DCs, which could result in a higher sensitivity to IDO-induced tryptophan depletion, maintaining the immune system in balance. However, in pathological conditions such as autoimmune disease, IDO-expressing cells lose the negatively regulative effects caused by tryptophan depletion because of autoreactive T cells expressing high levels of TTS, leading to the over-expansion of autoreactive T cells. Our findings certainly demonstrated this mechanism in GD patients.

In summary, our data provide evidence that the imbalance of immune system occurred in GD patients can be

caused by IDO- and TTS-mediated tryptophan metabolism disorder. We demonstrated that increased TTS expression in $CD4^+$ T cells acquired resistance to IDO-mediated tryptophan deprivation from DCs. IFN-γ brings into effect on modulating IDO and TTS expression. Given the importance of tryptophan metabolism in regulating T cell-mediated immune response, our findings provide a new insight into the pathophysiology and therapeutic approach for GD patients, meanwhile, also may offer a strategy to improve the efficacy of the therapy for GD patients in future through disrupting TTS expression in autoreactive T cells.

Acknowledgments We highly appreciate Dr. Imelda Lee for critical review of the manuscript. This work was supported by grants from the program of Health Bureau Shanghai Municipality (2006049) and Shanghai Commission of Science and Technology (08DZ2271000).

References

1. B. Rapoport, G.D. Chazenbalk, J.C. Jaume, S.M. McLachlan, Endocr. Rev. **19**, 673–716 (1998)
2. O. Saitoh, Y. Nagayama, Endocrinology **147**, 2417–2422 (2006)
3. B.S. Prabhakar, R.S. Bahn, T.J. Smith, Endocr. Rev. **24**, 802–835 (2003)
4. A.L. Mellor, D.B. Keskin, T. Johnson, P. Chandler, D.H. Munn, J. Immunol. **168**, 3771–3776 (2002)
5. M. Friberg, R. Jennings, M. Alsarraj, S. Dessureault, A. Cantor, M. Extermann, A.L. Mellor, D.H. Munn, S.J. Antonia, Int. J. Cancer **101**, 151–155 (2002)
6. D.H. Munn, M.D. Sharma, D. Hou, B. Baban, J.R. Lee, S.J. Antonia, J.L. Messina, P. Chandler, P.A. Koni, A.L. Mellor, J. Clin. Invest. **114**, 280–290 (2004)
7. U. Grohmann, F. Fallarino, R. Bianchi, C. Orabona, C. Vacca, M.C. Fioretti, P. Puccetti, J. Exp. Med. **198**, 153–160 (2003)
8. K. Sakurai, J.P. Zou, J.R. Tschetter, J.M. Ward, G.M. Shearer, J. Neuroimmunol. **129**, 186–196 (2002)
9. L. Zhu, F. Ji, Y. Wang, Y. Zhang, Q. Liu, J.Z. Zhang, K. Matsushima, Q. Cao, Y. Zhang, J. Immunol. **177**, 8226–8233 (2006)
10. J. Fleckner, P.M. Martensen, A.B. Tolstrup, N.O. Kjeldgaard, J. Justesen, Cytokine **7**, 70–77 (1995)
11. M.F. Murray, Lancet Infect. Dis. **3**, 644–652 (2003)
12. A. Boasso, J.P. Herbeuval, A.W. Hardy, C. Winkler, G.M. Shearer, Blood **105**, 1574–1581 (2005)
13. D.H. Munn, M.D. Sharma, J.R. Lee, K.G. Jhaver, T.S. Johnson, D.B. Keskin, B. Marshall, P. Chandler, S.J. Antonia, R. Burgess, C.L. Slingluff, A.L. Mellor, Science **297**, 1867–1870 (2002)
14. B. Widner, E.R. Werner, H. Schennach, H. Wachter, D. Fuchs, Clin. Chem. **43**, 2424–2426 (1997)
15. C.A. Williams, R.A. Harry, J.D. McLeod, Immunology **124**, 89–101 (2008)
16. A.L. Mellor, B. Baban, P. Chandler, B. Marshall, K. Jhaver, A. Hansen, P.A. Koni, M. Iwashima, D.H. Munn, J. Immunol. **171**, 1652–1655 (2003)
17. P. Terness, T.M. Bauer, L. Röse, C. Dufter, A. Watzlik, H. Simon, G. Opelz, J. Exp. Med. **196**, 447–457 (2002)
18. T. Hayashi, L. Beck, C. Rossetto, X. Gong, O. Takikawa, K. Takabayashi, D.H. Broide, D.A. Carson, E. Raz, J. Clin. Invest. **114**, 270–279 (2004)
19. A.L. Mellor, D.H. Munn, Nat. Rev. Immunol. **4**, 762–774 (2004)
20. Y. Kudo, C.A. Boyd, J. Physiol. **531**, 405–416 (2001)
21. M. Praetorius-Ibba, N. Stange-Thomann, M. Kitabatake, K. Ali, I. Söll, C.W. Carter, M. Ibba, D. Söll, Biochemistry **39**, 13136–13141 (2000)
22. C. Uyttenhove, L. Pilotte, I. Theate, V. Stroobant, D. Colau, N. Parmentier, T. Boon, B.J. Van den Eynde, Nat. Med. **9**, 1269–1274 (2003)
23. A. Curti, M. Aluigi, S. Pandolfi, E. Ferri, A. Isidori, V. Salvestrini, I. Durelli, A.L. Horenstein, F. Fiore, M. Massaia, M. Piccioli, S.A. Pileri, E. Zavatto, A. D'Addio, M. Baccarani, R.M. Lemoli, Leukemia **21**, 353–355 (2007)