Human Autoantibodies Modulate the T Cell Epitope Repertoire but Fail to Unmask a Pathogenic Cryptic Epitope

Sonia Quaratino, Jean Ruf, Mohamed Osman, Jin Guo, Sandra McLachlan, Basil Rapoport and Marco Londei

http://www.jimmunol.org/content/174/1/557

References
This article cites 50 articles, 23 of which you can access for free at:
http://www.jimmunol.org/content/174/1/557.full#ref-list-1

Subscription
Information about subscribing to The Journal of Immunology is online at:
http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Human Autoantibodies Modulate the T Cell Epitope Repertoire but Fail to Unmask a Pathogenic Cryptic Epitope

Sonia Quarantino, Jean Ruf, Mohamed Osman, Jin Guo, Sandra McLachlan, Basil Rapoport, and Marco Londei

Abs can tune the responses of Ag-specific T cells by influencing the nature of the epitope repertoire displayed by APCs. We explored the interaction between human self-reactive T cells and human monoclonal autoantibodies from combinatorial Ig-gene libraries derived from autoimmune thyroiditis patients and specific for the main autoimmune thyroid peroxidase (TPO). All human mAbs extensively influenced the T cell epitope repertoire recognized by different TPO-specific T cell clones. The action of the human mAbs was complex, because sometimes the same Ab suppressed or enhanced the epitopes recognized by the 10 different TPO-specific T cell clones. The human mAbs could modulate the epitope repertoire when TPO was added exogenously and when expressed constitutively on the surface of APCs. However, they could not unmask an immunodominant cryptic TPO epitope. In this study, we show that human autoantibodies influence the activity of self-reactive T cells and prove their relevance in concealing or exposing epitopes recognized by self-reactive T cells. However, our results further stress the biological significance of the immunodominant cryptic epitope we have defined and its potential importance in the evolution of autoimmunity. The Journal of Immunology, 2005, 174: 557–563.

The interaction between T and B cells has a key role in the fine-tuning of Ag-specific responses, and in the last few years, much work has been performed to unravel how this cross talk happens (1). T and B cells can communicate by exchanging information via the release of soluble factors or cognate interactions (2). One of the key functions of B cells is to present Ags to CD4+ Ag-specific T cells (3). During the evolution of an Ag-specific B cell immune response, the high-affinity BCR will allow the B cell to compete and overcome other types of professional APCs in capturing and presenting Ags to T cells (4). This characteristic is at the basis of Ab maturation, because B cells with higher affinity receptors will outcompete other B cells with lower affinity receptors for T cell help. Only B cells with higher affinity receptors will receive the optimal maturation signals from Ag-specific T cells (5). B cells can induce an anti-inflammatory response by presenting Ags, and in particular self-Ags, to T cells (6). By capturing Ags via their surface Ag receptor, they will change the type of Ag processing, and ultimately presentation of the T cell epitope repertoire available to T cells (5, 7). Abs, which are the soluble form of the BCR, can also influence T cell epitope availability, and several studies have elucidated some of the molecular mechanisms that control this occurrence (8–11).

T and B cell interactions play an intricate role in adaptive responses to foreign agents, such as bacteria and viruses, and in autoimmune diseases. In particular, in organ-specific autoimmune diseases such as type 1 diabetes and thyroiditis, both T and B autoantigen-specific responses are observed, and in most cases, they are specific markers of disease (12, 13). Although it is well recognized that B and T cells can cross talk, very little is known on how this might occur in humans, and in particular how high-affinity autoantibodies can influence the nature of epitope(s) recognized by the self-Ag-specific T cells. Although some studies have been performed in mouse models (14, 15) or in a mouse/human hybrid system (16), there is no report that addressed this question in human autoimmune diseases. This is because the appropriate tools to perform this study, such as a suitable combination of T cell clones and human monoclonal autoantibodies (mAbs) specific for the same autoantigen have not been available. The generation of human mAbs with overlapping functional characteristics of the autoantibodies detected in vivo has been a problem for these types of studies. Mouse mAbs, which are easier to generate, do not always have the same characteristics (epitopes or affinities) as human autoantibodies. This obstacle has been overcome by the Ig gene combinatorial library approach that permits the generation of human mAbs specific for foreign or self-Ags (17). Human mAbs specific for thyroid peroxidase (TPO),3 a dominant autoantigen in autoimmune thyroiditis (12) have been obtained by a gene combinatorial library (18). These Abs have the same characteristics (affinity and epitope recognition) of the natural polyclonal autoantibodies present in patients (19). We have previously generated and described a panel of well-characterized TPO-specific human self-reactive T cell

---

1 This work was supported by the Wellcome Trust (to S.Q.), European Community Grants BMH4-98-3703 (to S.Q.) and QLK1-CT-1999-00037 (to M.L.), and National Institutes of Health Grant DK36182 (to B.R.).

2 Address correspondence and reprint requests to Dr. Sonia Quarantino, Cancer Sciences Division, University of Southampton, Tremona Road, Southampton SO16 6YD, U.K. E-mail address: sq@soton.ac.uk

3 Abbreviations used in this paper: TPO, thyroid peroxidase; SI, stimulation index; DC, dendritic cell; MVA, modified vaccinia Ankara.
clones derived from thyroid autoimmune patients (20). They constituted the right tool to explore the interaction between autoantibodies and self-reactive T cells specific for the same autoantigen. These T cell clones were also instrumental in defining and characterizing a human cryptic epitope of TPO (21) involved in the development of autoimmunity (22). This cryptic epitope was presented when TPO was expressed as a surface molecule (21), as normally occurs in vivo on thyroid epithelial cells (23), but not when DC or other professional APC were loaded with exogenous TPO (21, 24).

The availability of these tools allowed us to address some basic questions on the nature of the interaction between T and B cells in a human autoimmune condition, but, more importantly, to explore whether these autoantibodies could unmask a bona fide and pathogenic human cryptic self-epitope. The results that we have generated indicate that human autoantibodies can modify the pattern of T cell reactivity of all 10 TPO-specific T cell clones. In contrast, only 1 of the 10 mouse anti-human TPO mAbs studied in parallel (25) exerted any activity on the epitopes recognized by the T cells clones. Despite their ability to widen the TPO T cell epitope repertoire, none of the tested human and mouse autoantibodies could unveil the immunodominant cryptic and pathogenic T cell epitope.

### Materials and Methods

#### T cell clones

Human T cell clones specific for different epitopes of the TPO molecules have been described previously (20, 21). They were established from the thyroid infiltrate of a patient with autoimmune thyroiditis and maintained at 1 × 10^7/well in RPMI 1640 supplemented with 10% human AB serum, 100 U/ml penicillin, and 50 μg/ml streptomycin (complete medium). Cells were fed at regular intervals with recombinant human IL-2 (20 ng/ml; kindly donated by Hoffman-La Roche) and stimulated every 3 wk with irradiated (4500 rad) allogeneic peripheral blood leukocytes and PHA.

#### T cell proliferation

The reactivity of the TPO-specific human T cells clones was assessed by coculturing 1 × 10^5 T cells from each T cell clone with either 3 × 10^9 glutaraldehyde-fixed autologous EBV-transformed B cell line or 1 × 10^5 well live HLA-matched human DC as APC. APC were incubated with TPO Ag or peptides with or without appropriate Abs for up to 5 h at 37°C before the addition of the T cells.

Human purified TPO Ag was used at 0.1 μg/ml. The peptide TPO_{164-167} (DPLIRGLALLPARPA) is a gift from J. D. D.Wraith (University of Bristol, Bristol, U.K.). All T cell clones were tested at least twice, and all of the experiments showed a similar profile of responsiveness. Stimulation index (SI) was calculated as follows: (total cpm − background cpm)/background cpm. All of the proliferative assays were performed in triplicate for 72 h in a round-bottom 96-well microtiter plate, and the cells were pulsed with [3H]thymidine (1 μCi) during the last 8 h of culture.

Human TPO-specific human and mouse Abs

Human TPO autoantibodies were isolated from Ig gene combinatorial libraries constructed from thyroid-infiltrating lymphocytes from patients with autoimmune thyroid disease (18). These recombinant autoantibodies were cloned and expressed as Fab molecules and have high affinity for TPO, with autoimmune thyroid disease (18). These recombinant autoantibodies were cloned and expressed as Fab molecules and have high affinity for TPO (K_d ~ 10^{-10} M). Four representative FabS (SP1.4, WR1.7, TR1.8, and TR1.9) were produced in bacteria, purified by affinity chromatography, and used at 3.2 × 10^{-8} M final concentration. These FabS define overlapping, conformal TPO epitopes recognized by all patients (18, 26). One of these autoantibodies (Fab SP1.4) was converted to a full-length human Ig of different isotypes (IgE, IgG1, and IgG4) of comparable levels of TPO binding (19). They were used at saturating concentrations equivalent to 1/100 for the IgG1, 1/25 for the IgE, and 1/1.5 for the IgG Abs. In addition to the human monoclonal autoantibodies, 10 mouse mAbs displaying different affinity for human TPO were generated as previously described (25) and were tested at 100 ng/ml for their ability to present TPO to T cell clones.

#### DC preparation

Human dendritic cells (DCs) were generated according to a well-established procedure (24, 27). Briefly, CD14^+ cells were isolated from 2 to 7 × 10^7 HLA-matched PBMC using magnetic cell separation system (Miltenyi Biotec). The CD14^+ cell fraction was then incubated for 5–7 days in presence of GM-CSF (20 ng/ml) and IL-4 (10 ng/ml) (R&D Systems). In vitro-generated DC were CD14^+, CD11c^+, and expressing high levels of MHC class II. DCs were further matured using TNF-α (10 ng/ml; a kind gift from the Centre of Molecular and Macromolecular Studies (Lodz, Poland)).

### Construction of recombinant modified vaccinia Ankara (rMVA) virus expressing TPO

Human TPO open reading frame was excised by cutting the plasmid pBSK-TPO with Xhol, filling the staggered ends with Klenow DNA polymerase, and further cutting with NotI. The resulting full-length cDNA encoding for the human TPO was ligated into the NotI site of pSC11 plasmid. The pSC11 plasmid carrying the human TPO gene was transfected with Perfect Lipid (Invitrogen Life Technologies). BHK21 cells were infected with MVA at 0.05 PFU per cell (MVA; a kind gift from Prof. G. Smith (Imperial College School of Medicine, London, U.K.).) Total virus from the cells and supernatant were harvested 3 days later and used for the reinfection of BHK21 cultures. The plaques of rMVA were identified by using X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) color selection and purified by five rounds of plaque purification (28). Bulk stocks of the rMVA were grown and purified by centrifugation of cytoplasmic extracts through a 36% (w/v) sucrose cushion in a Beckman SW28 rotor at 13,500 rpm for 80 min.

### Expression of TPO in DC and EBV-transformed B cell line

DC or EBV-transformed B cell lines were cultured at a density of 1 × 10^6/ml in a 24-well plate at 37°C in CO_2 and transduced with sonicated rMVA-TPO or rMVA-β-galactosidase (as negative control) at a multiplicity of infection of 10 as described (28). We used the MVA as a delivery vector to induce TPO surface expression, because we can easily infect both proliferating (like EBV-transformed B cell lines) and nonproliferating (like DC) cells (28).

TPO-transduced DC or the EBV-transformed B cell line were used as APC the following day. The analysis of the surface expression of TPO was performed between 4 h and 2 days postinfection. In some cases, the EBV-transformed B cell line was transfected with TPO encoded by the preEp4 vector as previously described (21, 29). In all cases, surface expression of TPO in the EBV-transformed B cell line and its APC activity produced overlapping results regardless of the method used to induce expression of the TPO.

### FACS analysis

Human DC were collected in ice-cold PBS (supplemented with 1% FCS and 0.05% azide) and stained using a panel of mouse anti-human TPO mAbs (IgG1) and a mouse anti-human IgG1 isotype control (Serotec). The autologous EBV-transformed B cell line was stained with FITC-conjugated mouse anti-human CD16 (clone 3G8), CD32 (clone AT10), CD64 (clone M22), and purified mouse anti-human CD23 (MHH6) (all raised in-house). The counterstain was a biotinylated rat anti-mouse IgG (Southern Biotechnologies, followed by PE-conjugated streptavidin (Southern Biotechnologies). Cells were then analyzed on a FACS (FACStarplus; BD Biosciences).

### Results

#### Human autoantibodies of the appropriate isotype capture the Ag and allow presentation by B cells

To evaluate whether human autoantibodies produce any modulation of T cell epitope repertoire, autologous EBV-transformed B cells were pulsed with human TPO or with TPO complexed to the human autoantibody SP1.4 either on the IgE or IgG4 backbones. Two hours later, the TPO-specific T cell clones were challenged with the different combinations of EBV, Abs, and Ag. As illustrated in Fig. 1A, the T cell clone 36 (representative of six other T cell clones) was not activated by the autologous EBV cell line pulsed with TPO but only by the TPO-expressing EBV cells (EBV-TPO). In contrast, a very powerful T cell response was observed when the T cells were stimulated with the EBV cell line pulsed with soluble TPO in the presence of saturating concentrations of the human mAb SP1.4 with an IgE (but not the IgG4) Fc region (Fig. 1B). The mAb SP1.4 on an IgG1 backbone tested at increasing concentrations behaved similarly to the SP1.4-IgE, but IgG4 always failed to induce a T cell response, even when tested...
at high concentrations (Fig. 1C). These observations demonstrate that the human autoantibody was efficient in binding the human TPO but only when the appropriate Ig backbone was used. It suggests that capture of the Ag/Ab complex, via the specific FcR, subsequently permitted Ag presentation by the EBV-transformed B cell line (30). The epitope recognized by T cell clone 36 is presented upon endogenous processing of TPO (TPO-EBV), and it is displayed following the piggyback action of the SP1.4 Ab. These data demonstrate that autoantibodies can capture a soluble autoantigen (TPO) and allow presentation of the autoantigen to self-reactive T cells.

Human, but not mouse, monoclonal autoantibodies can alter TPO presentation

We next wanted to assess whether a previously characterized panel of 10 mouse anti-human TPO IgG1 mAbs (25) had any ability to modulate TPO presentation to human T cell clones. These 10 mAbs were very efficient in detecting TPO expression in DCs transduced with the TPO gene delivered by rMVA virus (Fig. 2A). Similarly, all mouse Abs detected TPO expressed on the surface of the autologous EBV-transformed B cell line 4 h after rMVA-TPO transduction (data not shown). There was no specific TPO staining in cells transduced with the control virus rMVA-β-galactosidase (data not shown).

We then investigated what FcγRs were expressed by the autologous EBV-transformed B cell line by staining for CD16, CD32, and CD64. Of these, only CD32 (the FcγRII) is expressed by the autologous EBV-transformed B cell line (Fig. 2B). Furthermore, the FcεRII CD23 was also not expressed (data not shown).

In view of these results, we proceeded to address the effect of these mouse anti-human TPO Abs on TPO presentation to human T cell clones. Although all Abs detected surface expression of TPO in APC, only 1 (no. 15) of 10 mouse mAbs was able to allow TPO presentation to specific T cell clones (Fig. 3). The human autoantibody SP1.4, either on an IgG1 or IgE backbone, was very efficient in enhancing TPO Ag presentation by the EBV-transformed B cells to all of the six tested T cell clones (Fig. 3). Because all mouse anti-human TPO mAbs detected surface expression of TPO in both DC and EBV cell lines (Fig. 2A and data not shown), the failure of all but one of the mAbs to increase Ag presentation suggested that the epitope specificity of the Abs may play a key role in the Ag/epitope processing and presentation to T cells. This indicates that human high-affinity autoantibodies have a vast and unexpected potential to modulate Ag recognition by self-reactive T cells.

Human monoclonal autoantibodies modulate the epitope repertoire presented by APC expressing TPO as membrane-bound molecule

Because the human mAbs proved to be so efficient in permitting epitope availability to TPO-specific T cells (Fig. 1), our aim was to determine whether Abs could also modulate the loading of class II MHC-restricted T cell epitopes when the TPO is membrane bound.
TR1.9, and SP1.4, used at the saturating concentration of 10^8 M, used at the saturating concentration of 10^8 M, to avoid potential interference via FcR binding and piggyback effect, before adding the T cells.

as in thyrocytes (23). For this purpose, we tested four Abs, expressed as Fabs, that represent >80% of the human autoantibody repertoire (26) to assess whether they protect or footprint specific domains of the Ag during processing in EBV-TPO, that we have previously reported to stimulate selected TPO-specific T cell clones (21, 29).

In this experiment, the EBV-TPO cell line was coincubated for 2 h with the four human autoantibodies of different specificities expressed as Fabs, to avoid potential interference via FcR binding and piggyback effect, before adding the T cells. T cell clone 59 only marginally recognized TPO expressed by the EBV cell line, but in the presence of the Fab WR1.7, we observed a significant boost in T cell proliferation, suggesting that this Fab was able to modulate Ag processing operated by the TPO-EBV (Fig. 4). In contrast, none of the other tested Fabs seemed to have any function, even at high concentrations. However, each mouse anti-human TPO mAbs (IgG1) were tested simultaneously. Only mAb no. 15 was able to modulate the Ag processing by the EBV cell line used as APC. For simplicity, only three mouse mAbs are shown, because the others had no effect on T cells, as nos. 40 and 64.

The human autoantibodies do not allow the presentation of a cryptic epitope

We have so far described that human mAbs are highly efficient in modulating the T cell epitope repertoire. Therefore, we addressed the question whether Abs could unmask the cryptic TPO epitope recognized by pathogenic and immunodominant T cell clone 37 (21, 22). This TPO epitope is presented in vivo only upon endogenous processing in thyroid epithelial cells and in vitro in TPO-transduced cells such as EBV-transformed B cells (21). We have demonstrated that T cell clone 37 is not stimulated by fully competent DCs pulsed with TPO, but, on the contrary, it is anergized by a tolerogenic epitope presented by DCs (24).

We now tested whether the TPO epitope repertoire presented by DCs could be influenced by the human TPO-specific autoantibodies to display the cryptic epitope to T cell clone 37. As shown in Fig. 6A, T cell clone 37 failed to recognize the DCs pulsed with TPO but was only stimulated by its cognate TPO536–547 peptide, and none of the human autoantibodies could unmask this cryptic epitope when DCs presented TPO upon endogenous processing.

<table>
<thead>
<tr>
<th>Antigenic Challenge</th>
<th>Clone 23</th>
<th>Clone 58</th>
<th>Clone 59</th>
</tr>
</thead>
<tbody>
<tr>
<td>EBV-TPO</td>
<td>22</td>
<td>19</td>
<td>4.2</td>
</tr>
<tr>
<td>+ WR1.7</td>
<td>2.5</td>
<td>1.8</td>
<td>46.5</td>
</tr>
<tr>
<td>+ TR1.8</td>
<td>78</td>
<td>5.5</td>
<td>4.5</td>
</tr>
<tr>
<td>+ TR1.9</td>
<td>6</td>
<td>11.8</td>
<td>4.6</td>
</tr>
<tr>
<td>+ SP1.4</td>
<td>13</td>
<td>20.9</td>
<td>4.2</td>
</tr>
</tbody>
</table>

*Three TPO-specific T cell clones were challenged with the TPO-EBV with and without the four different human Fabs. Each Fab fragment (16 ng/ml) was coincubated with the TPO-EBV for 2 h prior to the addition of the T cells. SI was calculated as described in Materials and Methods. A SI < 3 is considered no proliferation in response to the specific trigger.*
In the same experiment, DCs presented the cognate TPO epitope to T cell clone 43 (21), and this epitope was significantly up-regulated by SP1.4 IgG1 (unpaired t test, \( p < 0.0001 \)) and down-regulated by all Fabs (\( p < 0.0001 \)).

DCs can strongly stimulate T cell clone 37 only when TPO is expressed as a surface molecule and presented by endogenous processing (Fig. 6B), confirming that regardless of the type of APC (epithelial cells, EBV-transformed B cells, or MVA-TPO-transduced DC), only endogenous processing allows the presentation of the cryptic self-epitope.

**Discussion**

In autoimmune diseases, the production of autoantibodies and the activation and expansion of self-reactive T cells is common. The close interaction between B and T cells in autoimmunity is demonstrated by the colocalization of CD4+ T cells and B cells in the affected tissue that may enable the B cells to present autoantigens to T cells and at the same time enable CD4+ T cells to provide help for autoantibody production (31).

In this complex interaction, Ags could be internalized through the B cell-specific membrane receptors and presented to helper CD4+ T cells at very low Ag concentrations. In this way, B cells can compete and outperform more professional APCs such as DCs (4). B cell Ag receptors, mannose receptors, and receptors for the Ig Fc region, promote both internalization and efficient presentation at low Ag concentrations. Thus, binding to specific membrane receptors concentrate Ags on APCs and mediates efficient uptake (30).

This has clear biological implications, because direct talk between T and B cells can influence the overall adaptive immune response to Ag (3, 32–34). It has been described that B cell Ag presentation to T cells will control the functional response of the Ag-specific T cells (35, 36). Recently, it has been also proven that B cells might release a different repertoire of cytokines and be subdivided into B effector type 1 or 2 (2). They can further influence the progression of autoimmune diseases by acting as regulatory cells (37, 38). In autoimmune diseases, B cells expressing the appropriate BCR can more avidly capture and present the Ag, thus reducing the opportunity for mature DC cells, which normally dictate a proinflammatory type of response (39).

Another way in which B cells can alter the T cell response is via the specific modulation of the epitope(s) displayed (8). Biochemical analysis demonstrated that both the suppressed and boosted epitope fall within an extended domain of Ag stabilized or footprinted by this Ab during proteolysis (8). Obviously, changes in the T cell epitope repertoire will totally modify the T cell response to foreign or self-Ags. This latter modification, contrary to the one in which B cells exert their function via the release of soluble factors, does not require that the Ag is presented by the same Ag-specific B cells, and indeed the soluble receptors (Abs) have been shown to modify the type of peptide presented to T cells (8, 40). It has even been postulated that Abs acting in this way could allow the unmasking of cryptic epitopes (5), which could have an extremely dangerous effect in the induction and perpetuation of autoimmunity (41).

Although cryptic epitopes, potentially involved in autoimmunity, have been described in mouse (42), they have been more difficult to detect in man. We have described one of these cryptic epitopes in human TPO, a dominant autoantigen in autoimmune thyroiditis (21). We defined that the pathway in which TPO is processed dictates the exposure of different epitopes recognized by self-reactive T cells (21, 43). Intriguingly, we have also described that this differential pattern of presentation can allow the display of tolerogenic peptides (24).

Although it has been suggested that autoantibodies could potentially alter the T cell epitope repertoire, no study in man has yet been performed, because the collection of appropriate autoantibodies and T cell clones to address this point have not been available. The results we have described above indicate that human monoclonal autoantibodies can alter the pattern of Ag presentation to self-reactive T cells. Biologically important conclusions have been generated by our study. The first is that the isotype of the Ab will significantly influence the Ag capturing and therefore the presentation of the conjugated Ag. For instance, in our study, we described that only Abs complexed to IgG1 or IgE could be captured by B cells, whereas the same Ab on an IgG4 backbone could not. This is because B cells only express FcγRII receptors, which can capture IgG1 (30) or IgE Igs (19). In keeping with these previous observations, the autologous EBV-transformed B cell line expressed neither CD16 nor CD64 (Fig. 2B) and was also negative for CD23 (data not shown). Thus, the isotype of the Abs will influence their action as modulator of Ag presentation. In this context, it has to be mentioned that polymorphisms of the FcRs may alter the ability to bind with different affinity to Igs, and this has been shown to have important biological consequences (44). More interestingly, Fc polymorphisms have been associated, at least in animal models, to predisposition to autoimmune diseases (45–47).

A very important outcome of our study was the finding that the ability to modulate Ag presentation was restricted to the human

---

**Table II. Enhancement and suppression of the TPO epitopes generated upon endogenous processing**

<table>
<thead>
<tr>
<th>T Cell Clones</th>
<th>12</th>
<th>27</th>
<th>39</th>
<th>40</th>
<th>58</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>SP1.4</td>
<td>0</td>
<td>↑</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>TR 1.9</td>
<td>0</td>
<td>↑</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>TR 1.8</td>
<td>↑</td>
<td>↑</td>
<td>0</td>
<td>0</td>
<td>↑</td>
<td>0</td>
</tr>
<tr>
<td>WR 1.7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Summary table for Fig. 5. ↑, Increase; ↓, decrease; 0, no change.
mAbs, whereas only one mouse anti-human mAb was able to affect TPO presentation by the autologous EBV-transformed B cell line. However, all of the mouse Abs recognized the human TPO as shown in Fig. 2, and their inability to influence Ag presentation could relate to their binding affinities, which may be lower than the human autoantibodies. Alternatively, they could recognize different epitopes not normally recognized by naturally occurring high-affinity human autoantibodies. Most of the TPO B cell epitopes are conformational, and thus the exact location has not yet been identified. The epitopes for mouse mAbs 18, 59, 64, and 15, and the human Fab SP1.4 and WT1.7 are within the A domain of TPO, whereas the epitopes for mAbs 9 and 47, and the human TR1.8 and TR1.9 are located within the B domain (48).

We have also used these mouse mAbs for similar studies with DC as APC, and still found them to be less efficient than the human monoclonals (data not shown). This further stresses the importance of performing these types of studies with the proper material. Our studies further stress the biological difference between mouse and human mAbs in the modulation of immune responses, suggesting that Ab binding specificities determine the effect on Ag processing.

Another important result of our study is that the human mAbs could interfere with the endogenous processing of TPO expressed on the surface of cells potentially acting as APC. The effects of the different Abs on the panel of T cell clones recognizing different epitopes of TPO was diverse, and depending on the combination of Abs and T cells, we could observe both up-regulation and down-regulation of T cell responses in agreement with other studies (8, 15). The fact that these autoantibodies can alter the pattern of endogenous recognition is of great biological significance, because TPO is expressed on the surface of thyroid epithelial cells (23), and these cells can in some cases act as APC (49, 50) and present TPO to T cells (20, 21).

In our previous studies, we have defined an immunodominant cryptic epitope generated by endogenous processing (21), recognized by 18% of the T cell clones infiltrating the thyroid (20). The analysis of the effects of the four human monoclonal autoantibodies clearly indicates that this epitope cannot be unmasked in DCs, even if the Abs are able to influence the epitope repertoire. However, we proved that DCs expressing TPO on their surface were able to present the cryptic epitope, excluding the possibility of an intrinsic inability of DCs to generate such an epitope. These data therefore reinforce the biological significance of the cryptic epitope we have defined. Although we cannot exclude that other human mAbs recognizing TPO might influence this recognition, this is unlikely. Indeed, the pattern of epitope recognition of human anti-TPO Abs is quite restricted, and the Abs used in this study appear to cover the whole repertoire of naturally occurring Abs (26).

In conclusion, we have demonstrated that human autoantibodies can alter the pattern of reactivity by human self-reactive T cells and further stress the dominance of the cryptic epitope we have previously described. The understanding of how autoantibodies can influence presentation of T cell epitopes and why they do not influence the cryptic epitope will hold important clues on the mechanisms controlling autoimmunity.

ACKNOWLEDGMENTS

We thank Tim Elliott and Anthony Antoniou for critical reading of the manuscript and helpful discussion, and Mark Cragg for the kind gift of the FcR mAbs.