T Cell Recognition of Self-Human Histocompatibility Leukocyte Antigens (HLA)-DR Peptides in Context of Syngeneic HLA-DR Molecules

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Summary

It has been suggested that self major histocompatibility complex (MHC) peptides bound to self MHC molecules may be involved in the intrathymic induction of self tolerance. We studied the antigenicity of synthetic peptides derived from the first domain of DRβ1*0101 chain in a DRβ1*0101 responder. We found that a peptide corresponding to residues 21–42 of the β chain could elicit the proliferation of autoreactive T cells. A T cell line (TCL-SUN) and 7 of 9 T cell clones (TCC) derived from TCL-SUN specifically recognized peptide 21–42 in the presence of APCs carrying the DRβ1*0101 allele. DRβ1*0101 positive APCs stimulated the TCCs in the absence of peptide, although the magnitude of the response was much lower than in cultures with peptide. This suggests that self DR1 molecules are continuously processed into peptides that are presented by the DR1 molecules on the surface of the cells. The data indicate that some T cells whose TCR binds to self MHC peptides presented by self MHC molecules are not deleted, although their ligand is continuously present. TCCs specific for peptide 21–42 presented in the context of DR1 were also stimulated by cells heterozygous for DRβ1*0301 and 1601, indicating that some DR peptide-specific autoreactive T cells participate in alloreactivity.

It is now widely accepted that recognition of self MHC molecules by T cells during and after T cell development is the driving force that shapes the available repertoire (1–5). Positive selection may operate on the basis of low affinity TCR-MHC interactions, whereas negative selection may eliminate only high affinity interactions (1). MHC molecules act as peptide binding molecules for presentation of foreign antigen to helper and cytotoxic T cells (6, 7). Most self proteins are processed and presented as peptides in the context of self MHC molecules (8). Such complexes have been implicated in both positive and negative selection in the thymus (2–5).

Recent evidence indicates that both class I and class II self MHC molecules are processed into peptides that can be presented in the context of other self class II molecules at the surface of APCs (9–13). Using a mAb that detects a complex formed by I-A^b with peptide Eα 56–73 of I-E^b, Rudensky et al. (12) have shown that the complexes of self MHC molecule and self peptide derived from another self MHC molecule are expressed on APCs in thymic medulla, mediating negative selection, e.g., deletion of the T cells recognizing the complex. However, there is also evidence that some self MHC peptide–reactive T cells escape elimination resulting in incomplete peripheral tolerance (14). In the mouse, both class I and II MHC peptides were shown to interact efficiently with class II molecules to induce an autoreactive T cell proliferative response (14). Such autoimmunogenic peptides appear to represent cryptic epitopes, i.e., determinants that are not optimally processed and presented by APCs.

We have attempted to establish whether peptides derived from the amino acid sequence of the β1 chain of HLA-DR can induce T cell autoreactivity. In this paper, we describe a T cell line and clones generated by in vitro immunization of peripheral blood mononuclear cells from a healthy donor, carrying the DRβ1*0101/DRβ1*1403 genotype, with a peptide corresponding to residues 21–42 of the DRβ1*0101 chain. This DR1 peptide is presented by the DR1 molecule, being recognized by T cells only on APCs that express the DRβ1*0101 gene. Autoreactive peptide–specific T cells also respond to the native DRβ1*0101 molecule on the surface of APCs, suggesting that the naturally processed form of peptide 21–42 is continuously presented by self DRβ1*0101 molecule.

Materials and Methods

Peptide Synthesis. Synthetic peptides corresponding to residues 1–20 (peptide 1), 21–42 (peptide 2), 43–62 (peptide 3), and 66–90 (peptide 4) of the DRβ1*0101 chain (15) were synthesized by the general solid-phase methods described by Merrifield et al. (16) using

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a peptide synthesizer (model 430A; Applied Biosystems, Inc., Foster City, CA). Peptides were purified by reverse-phase chromatography. Each peptide chromatographed as a single peak, showed the expected amino acid composition, and >95% purity. Peptides were dissolved at a concentration of 1 mg/ml in RPMI 1640 medium, and further diluted to appropriate concentrations with culture medium.

Generation of Peptide-specific T Cell Line and Clones. Responding cells were PBMC from a healthy male donor (SUN) whose HLA-DRβ1* genotype is DRβ1*0101/1403. PBMC at 10^6/ml were stimulated in 24-well plates (Costar, Cambridge, MA) with 10 μg/ml of peptide 2 in RPMI 1640 medium supplemented with 10% human serum (Sigma Chemical Co., St. Louis, MO), 2 mM L-glutamine, and 50 μg/ml gentamicin (Gibco, Grand Island, NY). rIL-2 was added at 10 U/ml 3 d after initiation of the culture. The cultures were subsequently fed every 3–4 d with medium containing 20 U/ml rIL-2, and restimulated every 14 d with peptide 2, using as APCs irradiated (3,000 rad) autologous PBMCs at 2×10^6/ml (10). A line that responded to peptide 21–42 was obtained after two stimulations, and was cloned by limiting dilution at 0.5 cells/well in medium containing this peptide (10 μg/ml), rIL-2 (20 U/ml), and autologous irradiated PBMC (5×10^4/well). Growing clones were expanded and restimulated under the same conditions as described for the line.

Proliferation Assays. Responding T cells (2×10^4/well) were stimulated with peptides (2.5 μg/ml) in the presence of 5×10^4 irradiated APCs in 96-well round bottomed microtiter plates (Costar). In some experiments, responding T cells were challenged only with irradiated PBMCs. After 48 h, the cultures were labeled with tritiated thymidine (0.5 μCi/well) and harvested 18 h later. [^{3}H]TdR incorporation was then measured in a liquid scintillation counter (LKB Betaplate; Pharmacia LKB Nuclear, Gaithersburg, MD). Mean cpm of triplicate cultures and the SD of the mean were calculated.

Antibody Blocking Assay. Blocking assays were performed using supernatants from mAbs L243 and W6/32 (American Tissue Culture Collection, Rockville, MD) which recognize framework determinants of HLA-DR and of HLA-class I antigens, respectively. Anti-DP, DQ, CD4, and CD8 antibodies (Becton Dickinson & Co., Mountain View, CA) were dialysed against medium and used at 1 μg/ml. mAb TM-903, generated in our laboratory, reacts with HLA-DRβ1*0301 and is negative with HLA-DRβ1*1601 (17). All mAbs tested were added to the cultures at the initiation of the proliferative assay.

HLA-typing. The HLA-DRβ1* genotypes of cell used in the assays were determined by genomic typing of in vitro amplified DNA with sequence-specific oligonucleotide probes (SSOP) for DRβ1* alleles, using PCR primers, and SSOP provided by the XI International Histocompatibility Workshop, Yokohama, Japan.

PCR Analysis of Vβ Gene Usage. Total RNA preparation was performed as described by Auffray and Rougeon (18) with some modifications as previously described (13). The RNA was reverse transcribed into cDNA with Moloney Murine Leukemia Virus reverse transcriptase (13). The conditions used for PCR and the sequences of Vβ and Cβ primers were described by Choi et al. (19). Briefly, 2 μl cDNA from individual samples were amplified in the presence of 0.3 μM of a Vβ specific primer and a 3′ Cβ primer, 2.5 U of Taq polymerase (Perkin Elmer Corp., Norwalk, CT), 200 μM dNTPs and PCR buffer containing 1.5 mM $MgCl_2$, 50 mM KCl, and 10 mM Tris-HCl (pH 8.4). The amplification was performed in a thermocycler (Perkin-Elmer Cetus Corp.) for 25 cycles: 95°C melting, 55°C annealing, and 72°C extension for 1 min each. The amplified products were separated on 2% agarose gel. 1 μg of HaeIII-digested $\phi \times 174$ DNA (Gibco, BRL) was run in parallel as molecular weight markers.

Table 1. *Response of TCL-SUN to DR1 Peptides*

APC	DR1 peptide residues	[^{3}H]TdR Incorporation (TCL-SUN)
+	Medium	8,796
−	21–42	239
+	1–20	9,321
+	21–42	40,372
+	43–62	9,082
+	66–90	9,268

TCL-SUN was tested for reactivity to peptide 21–42 (2.5 μg/ml) in triplicate cultures using 2×10^4 responding cells and 5×10^4 irradiated autologous APCs/well. Cultures were labeled after 48 h and harvested 18 h later. Results are expressed as mean cpm. SD is <10%.

Results

Generation of TCL[1]-SUN. In previous studies we have shown that T cells from both DRβ1*0101 positive and negative individuals respond to synthetic 20-mer peptides derived from the amino acid sequence of the first domain of the DRβ1*0101 chain (13). Peptide 2 (residues 21–42) was used in the present studies for in vitro immunization of PBMC from individual SUN carrying the DRβ1*0101 and DRβ1*1403 alleles. TCL-SUN was obtained after two cycles of stimulation. The line was tested for reactivity to peptide 2, and to peptides 1, 3, and 4, used as controls, in the presence of autologous APCs (Table 1). TCL-SUN exhibited strong blastogenic responses to peptide 2. The line also responded to autologous APCs without added peptide, although this reaction was about fourfold weaker than that observed when peptide 2 was added. The response to peptides 1, 3, and 4, was of the same order of magnitude as that observed in cultures with APCs alone, suggesting that reactivity to the specific immunogen does not reflect the response to impurities contained in the peptide preparation. Thus, TCL-SUN responds specifically to peptide 2 presented by autologous APCs.

To identify the MHC-restrictive element required for recognition of peptide 2, TCL-SUN was tested for reactivity to this peptide in the presence of APCs sharing one or none of the DR alleles with the responder (Table 2). APCs from unrelated donors carrying the DRβ1*0101 allele were capable of presenting peptide 2 to TCL-SUN, and of eliciting autoreactivity, with the same efficiency as autologous APCs. PBMCs expressing DRβ1*0102 could neither present the peptide nor serve as targets, although DRβ1*0102 differs from DRβ1*0101 by only two amino acid residues in the first domain of the β1 chain. APCs sharing the DRβ1*1403 gene

[1] *Abbreviations used in this paper:* TCC, T cell clone; TCL, T cell line.

Table 2. *MHC Restriction of Peptide Recognition by TCL-SUN*

Cell	DRβ1* Genotype of APC	[³H]TdR Incorporation (TCL-SUN)		
		APC alone	No peptide	Peptide 21–42
	No APC		354	310
1	DRβ1*0101/1403	124	8,796	37,366
2	DRβ1*0101/0301	193	9,079	38,669
3	DRβ1*0102/0901	133	264	225
4	DRβ1*1403/0901	284	257	268
5	DRβ1*0301/1601	203	19,573	20,250
6	DRβ1*0301/1601	110	13,600	15,144
7	DRβ1*0301/1101	54	64	75
8	DRβ1*1601/1301	38	1,301	1,425
9	DRβ1*1601/1201	153	391	362
10	DRβ1*1601/1101	165	300	234
11	DRβ1*1601/07	133	266	280

TCL-SUN was tested for reactivity to peptide 21–42 (2.5 μg/ml) in the presence of APCs carrying different DR alleles. The reactions were set up as described in Table 1.

with the responder elicited no stimulation in cultures with or without peptide 2. This indicates that the DRβ1*0101 molecule serves as a restrictive element and that it elicits autoreactivity.

TCL-SUN also reacted to APCs from two donors (cells 5 and 6) expressing the DRβ1*0301/1601 genotype in cultures without added peptide. These DR3/DR16 heterozygous APCs, however, were unable to present peptide 2, since no additional stimulation was observed when peptide 2 was added to the cultures. APCs expressing only one of these genes, i.e., either DRβ1*0301 or 1601, however, did not stimulate the response with or without added peptide. Hence, the stimulatory moiety recognized by TCL-SUN on the surface of DRβ1*0301/1601 heterozygous cells may be a structure that resembles the complex formed by peptide 2 with the DRβ1*0101 molecule.

Characterization of TCCs Derived from TCL-SUN. Recognition of peptide 2 in the context of DR1 molecule, and recognition of DR1 positive APCs without exogenous peptide, may be carried out by two different sets of T cells comprised in TCL-SUN. For this reason, we cloned the line by limiting dilution, and characterized nine of the resulting clones (Table 3). Seven clones responded both to DRβ1*0101 positive APCs without added peptide, and to peptide 2 presented by these APCs, in a pattern identical to that of the line. All seven clones displayed strong blastogenic responses when stimulated with DRβ1*0301/1601 heterozygous APCs. Similar to TCL-SUN, these clones did not react against cells expressing only DRβ1*0301 or DRβ1*1601. This indicates that they recognize a structure presented by the heterozygous cells, and that this ligand resembles the complex formed by peptide 2 with the DRβ1*0101 molecule.

The observation that these clones recognize DRβ1*0101 APCs without the addition of exogenous peptide suggests that peptide 2 emerges from the natural processing of the DR1 molecule and that it binds to the DR1 molecule per se. Since the blastogenic response to autologous APCs is about eightfold weaker in the absence than in the presence of peptide 2, it appears that the amount of naturally processed self-peptide that binds to MHC does not saturate all the antigen binding sites on the DR1 molecule.

Two other clones, E1B2 and E2B2, reacted strongly against DRβ1*0101 positive APC. These TCCs, however, showed no enhanced blastogenesis when peptide 2 was added. TCC E1B2 and E2B2 also failed to recognize the DRβ1*0301/1601 heterozygous APCs, supporting the concept that these APCs express a structure which resembles the peptide 2/DR1 molecule complex.

These results, therefore, indicate that TCL-SUN comprises two populations of T cells: one which recognizes the synthetic or the naturally processed form of peptide 21–42 in context of DR1; the other which recognizes the DR1 molecule, probably together with an unknown peptide. It is possible that the higher level of autoreactivity displayed by TCL-SUN to APCs alone compared with the clones, reflects the summation of autoreactivity to self DR1 plus unknown peptide, and self DR1 plus (naturally processed) DR1 peptide 21–42.

mAbs Blocking Studies. Study of the reactivity to peptide 2 displayed by TCL-SUN and by TCC derived from it, have

Table 3. *Reactivity of T Cell Clones*

TCCs	Peptide 21–42	DRβ1* Genotype of APCs					
		0101/ 1403	0101/ 0301	0301/ 1601	1403/ 0901	0301/ 1101	1601/ 1301
FB2	–	5,964	7,011	26,094	514	318	451
	+	45,570	48,303	27,358	470	241	445
FB3	–	3,615	3,871	11,556	270	219	208
	+	27,688	25,448	11,911	316	148	140
FB4	–	3,449	3,820	23,735	395	564	463
	+	28,891	31,202	23,166	544	401	363
FC2	–	3,639	3,442	20,506	200	234	369
	+	21,564	18,998	19,857	263	109	413
FC4	–	6,518	6,350	26,353	444	580	503
	+	49,972	49,020	31,387	596	526	811
FC5	–	5,688	5,193	24,064	457	494	647
	+	43,627	45,106	27,322	518	479	432
E1C2	–	3,322	3,318	16,895	322	333	323
	+	25,939	25,052	18,755	469	550	337
E1B2	–	13,392	13,151	375	412	290	250
	+	13,218	13,926	368	285	363	366
E2B2	–	13,629	16,491	468	302	118	722
	+	16,463	18,880	480	675	248	360

TCCs were tested for reactivity to peptide 21–42 (2.5 μg/ml). The reactions were set up as described in Table 1.

revealed that the peptide is presented on the DR1 molecule. To confirm the observation that the response is HLA-DR restricted, we tested the reactivity of TCL-SUN and of one of the clones (FC4) to the peptide and to autologous APCs in the presence of mAb anti-HLA-class I and class II, and anti-CD4 and CD8. mAb to HLA-DR and to CD4 significantly inhibited the TCL and TCC response induced by autologous APCs with and without peptide. mAbs to HLA-DQ, DP, class I, and CD8 had no effect (Fig. 1).

In an attempt to establish whether the ligand recognized by TCL-SUN on the DRβ1*0301/1601 heterozygous cells consists of a DR3 molecule plus peptide, or DR16 molecule-plus-peptide complex, we used mAb TM-903 which reacts

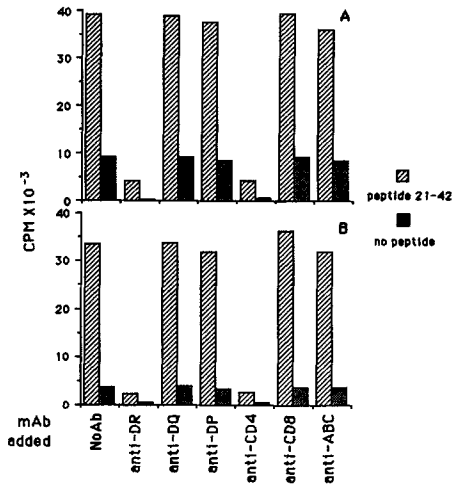

Figure 1. Effect of mAbs on proliferative responses of TCL-SUN and TCC-FC4. Autologous APCs (5 × 10⁴/well) were cultured with TCL-SUN (*A*) and TCC-FC4 (*B*) (2 × 10⁴/well) in the presence (▨) or in the absence (■) of peptide 21–42 (2.5 μg/ml). mAbs were added at the initiation of the cultures. Results are mean cpm of triplicate cultures. SD to the mean is <10%.

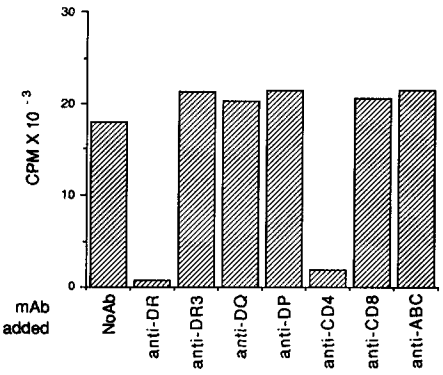

Figure 2. Effect of mAbs on the proliferative response of TCL-SUN to DR16/DR3 heterozygous cells. TCL-SUN (2 × 10⁴/well) was stimulated with DR16/DR3 heterozygous cells (5 × 10⁴/well) in the presence of mAbs. Results are expressed as mean cpm. SD to the mean is <10%.

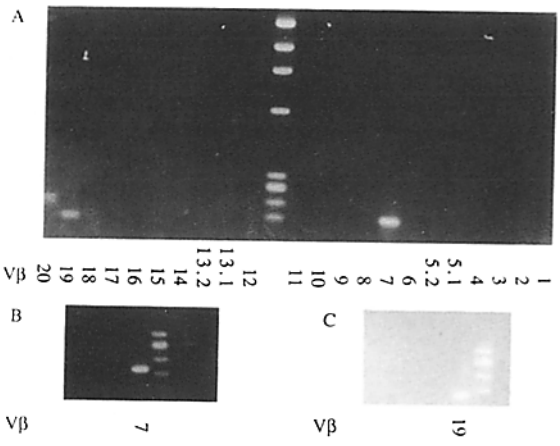

Figure 3. Amplification of cDNAs from TCL-SUN, TCC-FC4, and E1B2. RNAs from TCL-SUN (*A*), TCC-FC4 (*B*), and TCC-E1B2 (*C*) were reverse transcribed, PCR amplified and electrophoresed on 2% agarose gel. 1 μg HaeIII-digested $\phi \times 174$ DNA was run in parallel as molecular weight markers.

with DRβ1*0301, but not with DRβ1*1601 positive cells in blocking experiments (17). The response was inhibited by mAb to a common structure determinant of HLA-DR, but not by the anti-DR3 mAb TM-903 (Fig. 2). This suggests that reactivity is triggered by a DR16-plus-peptide rather than DR3-plus-peptide complex. Since both the DR16 and DR3 antigens, however, are required for stimulation to occur, it is possible that the peptide itself derives from DR3, or from the product of a linked gene.

Vβ Gene Expression in TCL-SUN and TCCs. To establish the clonality of the cells that recognize peptide 21–42 we studied the TCR-Vβ gene usage in TCL-SUN and in each of the nine clones derived from it. Complete analysis of the expression of mRNA for 20 families of human TCR-Vβ genes showed three bands corresponding to Vβ7, Vβ19, and Vβ20 in TCL-SUN (Fig. 3 *A*). The strongest message corresponded to Vβ7. All seven clones that recognize peptide 2 in context of DRβ1*0101 showed a single band corresponding to Vβ7 as illustrated in Fig. 3 *B*. The two clones with reactivity against autologous APCs, but not against the peptide, expressed Vβ19 (Fig. 3 *C*). Therefore, the response to self HLA-DR1 and to self-HLA-DR1-plus-peptide 21–42 is the function of distinct TCRs.

Discussion

The T cell repertoire is shaped by both positive and negative selection (1–5). Self peptides are not expected to be immunogenic because of the deletion of T cells recognizing self peptide/MHC complexes during intrathymic development. It has recently been shown that a self I-E^b peptide can be eluted from self I-A^b molecules (20), and that the I-E^b peptide is immunogenic for I-A^{b+}, I-E$^-$ mice, but not for I-A^{b+}, I-E^{b+} mice (12). These results suggest that T cell recognition of self MHC peptides expressed on the cell surface in association with self MHC molecules leads to the elimination of T cells recognizing such complexes. On the other hand, the existence of autoreactive T cells that recognize self MHC peptide/MHC molecule complexes has also been documented, although it was not proven that such complexes were naturally expressed on APCs (14).

The data presented in this paper demonstrate that autoreactive human T cells, which are specific for a self DR1 peptide/self DR1 molecule complex, escape negative selection, in spite of the fact that the complexes are expressed on peripheral APCs. We studied the antigenicity of a peptide corresponding to residue 21–42 of the DRβ1*0101 chain using this peptide for in vitro immunization of T cells from an individual carrying this allele. T cell lines and clones specific for the DR1 peptide 21–42 presented by DRβ1*0101 positive APCs were obtained. These lines were stimulated, although to a lesser degree, by DRβ1*0101 positive APCs even in the absence of the peptide. This suggests that self DR1 molecules are continuously processed and presented on the surface of APCs, raising questions about mechanisms suppressing and/or preventing the activation of anti-self MHC-reactive T cells.

Rudensky et al. (12) have suggested that differential expression of self peptide/MHC molecule complexes on thymic APCs may be a key feature for positive and negative selection. In this context, it is possible that autoreactive T cells, such as those described in our studies, have escaped tolerance induction because the DR1 peptide/DR1 molecule complexes which they recognize, are not expressed on the thymic APCs involved in negative selection. This mechanism, however, does not explain why such potentially autoreactive T cells are not activated in the periphery where the DR1 peptide is presented by APCs. Since DR1 positive APCs become more stimulatory when exogenous DR1 peptide is added, it is possible that the level of expression of DR1 peptide/DR1 molecule complexes in the thymus is too low for tolerance induction. For the same reason, DR1 peptide-specific T cells in the periphery may not be activated under normal conditions.

On the other hand, Benichou et al. (14) have demonstrated that some self MHC peptides interact efficiently with self MHC-class II molecules, eliciting autoreactive T cell proliferative responses, while others can bind but fail to stimulate reactivity. These authors suggest that there is a hierarchy of dominance among T cell determinants resulting from the processing of complex proteins (21, 22). T cells recognizing dominant determinants of self proteins, including self MHC molecules, are likely to be made tolerant during intrathymic development, while T cells specific for cryptic determinants could escape negative selection (14). Peptide 21–42 of DR1 may represent such a cryptic or subdominant determinant of the DR1 molecule which is probably not optimally processed and presented.

It has also been suggested that self peptide/MHC complexes at levels sufficient to activate naive CD4 T cells are of very limited complexity (12). By analogy, peptide 21–42 may be a dominant determinant of the DR1 molecule which fails to compete efficiently for binding to self DR1 molecules with dominant determinants of other self proteins. This possibility, as well as the hypothesis that peptide 21–42 corresponds to a cryptic epitope, implies that this self DR1 peptide/DR1 molecule complex occurs at a level that is suboptimal

for tolerance induction in the thymus, and for T cell activation in the periphery.

It is possible that the failure to eliminate all self-reactive T cells prevents excessive purging of the T cell repertoire by the immune system (14). Some self MHC peptide–reactive T cells may have a physiologic role recognizing certain pathogens by molecular mimicry. Such a mechanism may also account for alloreactivity, since complexes of allogeneic MHC plus peptide can mimic those formed by self MHC with peptide (23). Our finding that DR1 peptide-specific autoreactive T cells also recognized allogeneic MHC-plus-peptide complexes (presumably presented by the DRβ1*0301/1601 heterozygous cells) supports this notion.

The existence of potentially autoreactive T cells in the periphery as documented in our study, supports the hypothesis that such cells can be involved in autoimmune reactions under conditions of upregulation of MHC expression. Since the TCR-Vβ gene usage of autoreactive T cells is quite limited, the potential usefulness of antiidiotypic therapy for downregulation of autoaggressive clones can be envisioned.

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