Selective Modulation of CD4$^+$ T Cells from Lupus Patients by a Promiscuous, Protective Peptide Analog[1]

Fanny Monneaux,* Johan Hoebeke,* Christelle Sordet,† Céline Nonn,‡ Jean-Paul Briand,* Bernard Maillère,‡ Jean Sibillia,† and Sylviane Muller[2]*

A peptide encompassing residues 131–151 of the spliceosomal U1-70K protein and its analog phosphorylated at Ser^{140} were synthesized as potential candidates for the treatment of patients with lupus. Studies in the MRL/*lpr* and (NZB × NZW)F$_1$ lupus models have demonstrated that these sequences contain a CD4$^+$ T cell epitope but administration of the phosphorylated peptide only ameliorates the clinical manifestations of treated MRL/*lpr* mice. Binding assays with soluble HLA class II molecules and molecular modeling experiments indicate that both peptides behave as promiscuous epitopes and bind to a large panel of human DR molecules. In contrast to normal T cells and T cells from non-lupus autoimmune patients, we found that PBMCs from 40% of lupus patients selected randomly and CFSE-labeled CD4$^+$ T cells proliferate in response to peptide 131–151. Remarkably, however, we observed that phosphorylation of Ser^{140} prevents CD4$^+$ T cells proliferation but not secretion of regulatory cytokines, suggesting a striking immunomodulatory effect of phosphorylated analog on lupus CD4$^+$ T cells that was unique to patients. The analog might act as an activator of regulatory T cells or as a partial agonist of TCR. ***The Journal of Immunology*****, 2005, 175: 5839–5847.**

Besides intrinsic dysfunctions of B lymphocytes that characterize systemic lupus erythematosus (SLE),[3] numerous abnormalities also affect T cell response, the production of T cell cytokines, and the T-B cell dialogue. T cells appear to have a central role in the pathogenesis of SLE. T cells that are oligoclonal based upon TCR usage have been shown to infiltrate the kidneys of lupus patients (1) and expansion of oligoclonal T cells in peripheral blood of patients has been demonstrated (2). Th cells are essential for sustaining autoantibody-producing B cells and it is widely believed in lupus that certain Th cell subsets drive B cells to produce pathogenic autoantibodies. CD4$^+$ T cell epitopes of a number of nuclear Ags have been characterized in human lupus as well as in mixed-connective tissue disease and Sjögren's syndrome using either short-term T cell lines, T cell clones and cloned Th hybridomas, or PBMC (2–7). By testing a series of overlapping peptides, we previously identified an epitope present in residues 131–151 of the spliceosomal U1-70K small nuclear ribonucleoprotein, recognized very early by IgG Abs and CD4$^+$ lymph node T cells from both H-2^k MRL/*lpr* and H-2$^{d/z}$ (NZB × NZW)F$_1$ lupus-prone mice (8, 9). Fibroblasts transfected with MHC class II molecules were used to demonstrate that peptide 131–151 binds I-A^k, I-E^k, I-A^d, and I-E^d murine MHC molecules (8, 9). We further showed that an analog of this sequence phosphorylated on Ser^{140} (named peptide P140) was strongly recognized by lymph node and peripheral CD4$^+$ T cells and by IgG Abs from MRL/*lpr* mice (10, 11). This analog and the cognate peptide 131–151 were used in therapeutic trials in lupus-prone mice to investigate their ability to restore tolerance. Young MRL/*lpr* mice were given the peptides i.v. in saline and we found that P140 peptide but not the parent peptide 131–151 reduced proteinuria and dsDNA IgG Ab levels and significantly enhanced the survival of treated mice (10). In contrast, administrated s.c. in Freund's adjuvant, P140 peptide accelerated lupus nephritis. Identification of a tolerogenic CD4$^+$ T cell epitope in P140 peptide is remarkable because this sequence, which is completely conserved in the mouse and human U1-70K protein, contains an RNA-binding motif called RNP1 also present in other small nuclear/heterogeneous nuclear ribonucleoproteins and often targeted by Abs from lupus patients and mice. We proposed a model in which the RNP1 motif could initiate the spreading of the immune response to the whole U1-70K protein and then sequentially to other proteins present in the same spliceosomal particle containing or not the RNP1 motif by an intermolecular spreading mechanism (12, 13). We recently demonstrated experimentally that an intramolecular T and B cell spreading effectively occurs in MRL/*lpr* mice tested at different ages (11, 12). Moreover, we showed that repeated administration of phosphorylated analog P140 in saline into preautoimmune MRL/*lpr* mice transiently abolishes T cell intramolecular spreading to other regions of the U1-70K protein (11). Taken together, these findings suggest that as described in the case of histones in the SNF1 mouse model (14), the P140 analog might originate a mechanism of so-called "tolerance spreading" that leads to the beneficial effect observed in P140-treated MRL/*lpr* mice.

Prompted by these promising results in murine models of lupus, we investigated the spontaneous responsiveness and cytokine production of PBMCs from patients with SLE and from control subjects including patients with other systemic autoimmune diseases

*Centre National de la Recherche Scientifique (CNRS), Unité Propre de Recherche 9021, Institut de Biologie Moléculaire et Cellulaire, Strasbourg, France; †Département de Rhumatologie, Hôpital de Hautepierre, Strasbourg, France; and ‡Commissariat à l'Energie Atomique Saclay, Département d'Ingénierie et d'Etudes des Protéines, Gif-sur-Yvette, France

Received for publication May 25, 2005. Accepted for publication August 16, 2005.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported in part by CNRS, BioDelivery Systems and the French Association de Recherche sur la Polyarthrite. F.M. was supported by a short-term grant from the French Fondation pour la Recherche Médicale.

[2] Address correspondence and reprint requests to Dr. Sylviane Muller, Institut de Biologie Moléculaire et Cellulaire, UPR9021, CNRS, 15 rue René Descartes, 67084 Strasbourg, France. E-mail address: S.Muller@ibmc.u-strasbg.fr

[3] Abbreviations used in this paper: SLE, systemic lupus erythematosus; APL, altered peptide ligand; ANA, anti-nuclear Ab; SLEDAI, SLE disease activity index; ENA, extractable nuclear Ag.

0022-1767/05/$02.00

in response to peptides 131–151 and P140. We also analyzed HLA restriction elements used in recognition of these two peptides by T cells, and to support our experimental findings, we modeled both analogs within HLA-DR molecules and studied their mode of binding in silico. The data described herein show that ex vivo as well as in silico, the phosphorylated analog P140 behaves as a "universal" epitope. In a manner that was nonexpected from experiments in murine models, we found in humans that the P140 analog is recognized frequently and selectively by lupus T cells and alters their functions. It could act either as an altered peptide ligand (APL) of the TCR or by stimulating $CD4^+/CD25^+$ regulatory T cells. These findings are of first importance in our quest for specific and selective treatments of autoimmune patients.

Materials and Methods

Patients

Blood samples were obtained from a total of 69 individuals, namely 34 unselected, consecutive patients with SLE, 27 patients with autoimmune diseases (rheumatoid arthritis, primary Sjögren's syndrome, autoimmune deafness, polymyositis, primary biliary cirrhosis, autoimmune hepatitis), 4 patients hospitalized for nonautoimmune or infectious diseases, and 4 normal individuals. All autoimmune patients fulfilled the American College of Rheumatology criteria for these respective diseases. They were recruited from the Strasbourg University Hospital Center of Hautepierre after informed consent was obtained. Patients were treated by low doses (median dose 10 mg; range 0–20 mg) of methotrexate, hydroxychloroquine, and/or nonsteroidal anti-inflammatory drugs (Table I). Excluded from the series of patients enrolled in this study were all patients who received prolonged and heavy suppressive treatments. All but 1 of the 34 SLE patients (range 15–81 years, median 34.08 years; 31 females and 3 males) had anti-nuclear Abs (ANA; titers 80–1280 IU/ml as measured by immunofluorescence on Hep-2 cells), 14 of 34 had elevated levels of anti-dsDNA Abs as measured by two independent ELISA kits (Pharmacia; Kallestad), and 20 of 34 possessed extractable nuclear Ag (ENA) as measured by double immunodiffusion (Ouchterlony) and dot-blotting (D-Tek). Disease activity was determined according to the SLE disease activity index (SLEDAI) score (15). DNA-based HLA-DRB and DQB typing (Micro SSP generic HLA class II kit; One λ; InGen) was available for all. All but 6 of 27 patients with autoimmune diseases other than SLE (range 33–83 years; median 52 years; 25 females and 2 males) had ANA, one had low titers of anti-dsDNA Abs, 12 possessed ENA, and 2 had anti-mitochondria Abs.

Peptides

Peptides were synthesized using classical F-moc (*N*-[9-fluorenyl] methoxycarbonyl) solid-phase chemistry on a home-made, fully automated multichannel peptide synthesizer (10).

HLA-DR peptide-binding assays

Peptide-binding assays to HLA-DR molecules were performed as described previously (16) using DR molecules purified from EBV homozygous cell lines by affinity chromatography (17, 18). The biotinylated peptides used as competitors were the following: HA 306–318 for DRB1*0101 (1 nM (pH 6)), DRB1*0401 (30 nM (pH 6)), DRB1*1101 (20 nM (pH 5)), and DRB5*0101 (10 nM (pH 5.5)); YKL for DRB1*0701 (10 nM (pH 5)); A3 152–166 for DRB1*0301 (200 nM (pH 4.5)); B1 21–36 for DRB1*1301 (200 nM (pH 4.5)); LOL 191–210 for DRB3*0101 (10 nM (pH 5.5)); and E2/E168 for DRB4*0101 (10 nM (pH 5)).

Molecular modeling

Four HLA-DR molecules, whose x-ray structures are known, were used as matrix for molecular modeling. They were HLA-DRB1*0101 (Ref. 19; Protein Data Bank (PDB 1FYT), HLA-DRB1*1501 (Ref. 20; PDB 1BX2), HLA-DRB1*030101 (Ref. 21; PDB 1A6A), and HLA-DRB1*0401 molecules (Ref. 22; PDB 1J8H). To check binding of peptide 131–151, amino acid replacement was manually performed by fixing a compatible amino acid residue at the pocket 1 position and sequentially replacing the other amino acid residues on the bound peptide. To avoid modeling artifacts, the manual replacement was only followed by a conjugate gradient minimizing procedure on the fixed HLA-DR molecule to check whether all the amino acid positions were compatible with binding. Only the models in which the noncovalent binding energy of the peptide was negative were retained. The programs used were, respectively, Biopolymer and Discover using consistent valence force field potentials from Accelrys. The models obtained with peptide 131–151 were subsequently phosphorylated in silico at Ser^{140} and a new round of conjugate gradient minimizing was performed to check the compatibility of this phosphorylation with binding. Finally, the models were charged at a pH of 7.4 and a new round of conjugated gradient minimization was performed using a distance-dependent dielectric constant.

Table I. *Characteristics of the first series of patients with systemic lupus erythematosus*[a]

Patient/Sex/ Age/Race $n = 22$	Disease Duration (years)	SLEDAI[b]	ANA (IIF)[c]	ENA (Ouchterlony)	Anti-dsDNA[d]	Organ Involvement	Therapy
Ban/F/36/C	25	14	640	RNP, Ro, La	<90	Joints, skin, Raynaud	MTX, CS, Inf
Ben/F/33/M	5	35	640	−	>300	Joints, skin, kidney, CNS, APLS	MTX, CS, HCQ
Blo/M/81/C	19	21	−	RNP	110	Joints, kidney, serositis, APLS	HCQ
Bou/F/43/C	4	11	320	Ro	110	Joints	HCQ, CS, MTX
Bri/F/29/C	3	10	640	−	>300	Joints, skin	HCQ, CS
Cha/M/30/M	8	11	1280	RNP, Ro	>300	Joints, skin	CS
Did/F/26/C	5	15	1280	Ro, La	300	Joints, skin	NSAID
Dol/F/15/C	3	13	1280	RNP	<90	Joints	MTX, CS
Fil/F/41/C	8	21	640	−	<90	Joints, skin, nevritis, APLS	None
Gio/F/22/C	5	9	320	−	<90	Joints, skin	HCQ
Gru/F/68/C	7	7	640	−	<90	Joints, skin, APLS	CS
Izo/F/20/C	6	28	1280	RNP	105	Joints, skin, nevritis	HCQ, CS
Lac/F/50/C	16	11	1280	RNP	>300	Joints, skin, Raynaud	HCQ, CS
Laz/F/30/C	8	9	1280	Ro	297	Joints, skin	HCQ, CS
Le/F/38/A	9	13	1280	Ro	200	Joints, skin	HCQ
Pel/F/20/C	7	9	320	−	<90	Joints	HCQ
Peu/F/27/A	6	16	640	RNP	246	Joints, skin, serositis	HCQ
Piq/T/29/M	8	8	1280	RNP	<90	Joints, Raynaud	HCQ, CS, MTX
Sch/F/41/C	8	7	1280	Ro, La	110	Joints, skin	HCQ
Scho/F/18/C	2	6	1280	Ro, La	nd	Joints, skin	NSAID
Tua/F/31/C	8	10	320	−	<90	Joints	HCQ
Woz/F/36/C	8	11	1280	−	>300	Joints, skin	HCQ

[a] Abbreviations used: C, Caucasian; M, Maghrebian; A, Asian; APLS, anti-phospholipid syndrome; MTX, methotrexate; CS, corticosteroids; HCQ, hydroxychloroquine; Inf, Infliximab; NSAID, nonsteroidal antiinflammatory drugs; nd, not done; −, negative.

[b] SLEDAI scores <15 were considered as weak.

[c] ANA were considered positive when the titer was ≥80 IU/ml as measured by IIF.

[d] Anti-DNA Abs were considered positive when the titer was ≥90 IU/ml as measured by ELISA.

The DRB5 model was constructed by changing manually the allelic variations of the B chain in the DR1 model. Subsequently, peptides 131–151 and P140 were introduced and minimized as for the other DR models.

Proliferation assay

PBMCs were isolated from 40 ml of fresh heparinized blood by centrifugation on Ficoll-Histopaque (Sigma-Aldrich). After washing and counting, cells were cultured in flat-bottom 96-well plates (5×10^5 cells/well) in the presence of increased concentrations of peptide (100 μl/well). All assays were performed in RPMI 1640 medium (Biomedia) containing 10% FCS (Biomedia), 1 mM HEPES, 10 μg/ml gentamicin, and 5×10^{-5} M 2-ME. Cells were incubated for 7 days at 37°C in a 5% CO_2 humidified atmosphere. The cultures were pulsed during 18 h with tritiated thymidine (1μCi/well; 6.7mCi/mmol; ICN) and DNA-incorporated radioactivity was measured using a Matrix 9600 direct β counter (Packard Instrument). The results are expressed as the arithmetic mean of thymidine uptake expressed as cpm. Proliferative responses were considered to be positive if the [^{3}H]thymidine uptake was equal to or above three times the uptake by cells cultured in medium alone without peptide. The SD of triplicate cultures was always below 20% of the mean. Control tests were performed by adding Con A (0.5 μg/well; Sigma-Aldrich) to cells during the time of the culture (7 days). Tests were set up in duplicate or triplicate. For the detection of IFN-γ, IL-4, and IL-10, culture supernatants (50 μl) were collected after 24 h and tested in a double-sandwich ELISA (BD Pharmingen). In some experiments, mAbs against HLA-DR (clone L243), HLA-DQ (clone SPVL3), and HLA-DP (clone B7/21) were added in the cultures at the final concentration of 1 μg/ml each.

Cell proliferation by CFSE tracking

Cells were suspended at 15×10^6/ml in RPMI 1640 without FCS. CFSE (FluoProbes; Interchim) was added v/v to cells at 2 μM for 10 min at 37°C in the dark. The reaction was stopped by adding an equal volume of FCS, followed by a 2-min incubation at room temperature. After two washes, CFSE-labeled cells were suspended in assay medium and cultured at 37°C and 5% CO_2 in the presence or absence of peptide (30 μM). After 8 days, cells from corresponding wells were pooled, washed with PBS/FCS, and stained with allophycocyanin-conjugated anti-CD4 mAb (RPA-T4; BD Pharmingen) for 15 min at 4°C. After washing, cells were acquired on a two-laser FACSCalibur (BD Biosciences) and analyzed using CellQuest software (BD Biosciences). At least 50,000 cells were collected, and gating was set by forward and side scatter.

Statistical analyses

Statistical analyses were made using the Student *t* test to compare between sample groups. Statistical significance of the data was set at $p < 0.05$.

Results

PBMCs from lupus patients proliferate ex vivo in the presence of peptide 131–151

The proliferative response of fresh PBMCs to peptide 131–151 (RIHMVYSKRSGKPRGYAFIEY) and to its phosphorylated analog P140 (RIHMVYSKRS(P)GKPRGYAFIEY) as well as to the two respective scrambled peptides Sc (YVSRYFGSAIRHEPK-MKIYRG) and ScP (YVSRYFGS(P)AIRHEPKMKIYRG) used as controls was measured individually in 22 unselected, consecutive patients with lupus and in 21 patients with other autoimmune diseases (P in parentheses indicates that serine (S) is phosphorylated). The clinical and biological characteristics of lupus patients including the SLEDAI and the treatment of lupus patients at the time the blood samples were collected are summarized in Table I. T cells from all patients in the SLE group and the group of patients with other autoimmune diseases showed a proliferative response to Con A. As shown in Fig. 1*A*, PBMCs from 8 of 22 unselected SLE patients (36%) and 1 of 21 patients with other systemic autoimmune diseases (5%; $p = 0.01$) proliferated ex vivo in the presence of 15 μM of peptide 131–151. This proliferative response was dose-dependent (shown in Fig. 1*B* with four representative examples). It was peptide 131–151-specific as demonstrated with the scrambled peptide Sc that induced proliferation of PBMCs at the limit of positivity only in the case of 2 of 22 SLE patients (Tua and Fil; $p = 0.03$; Fig. 1*A*). No significant correlation was found between the spontaneous proliferative responses of lupus patients' PBMCs measured ex vivo and their clinical/biological characteristics. It is notable, however, that among the eight positive patients (six females/two males), five (Ban, Bou, Cha, Pel, Tua) had a low SLEDAI score below 15, four (Ban, Fil, Pel, Tua) had no anti-dsDNA at the time their PBMCs were tested, and three (Fil, Pel, Tua) had no ENA as measured by the double-immunodiffusion assay of Ouchterlony. The patient Fil received no treatment, while the other 131–151 peptide-positive patients received mild treatments. Importantly, the P140 analog induced no proliferation of patient's PBMCs (Fig. 1*B*).

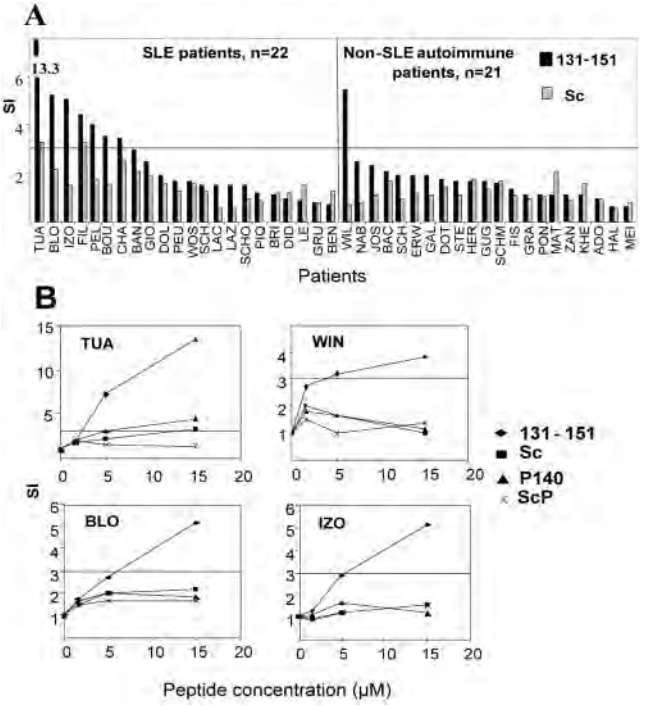

FIGURE 1. Peptide 131–151 of the U1-70K protein induces proliferation of PBMCs from SLE patients. *A*, Responses in a 7-day proliferation assay to peptides 131–151 (■) and Sc (▨) tested at 15 μM in 22 patients with SLE and 21 patients with other related autoimmune diseases. Results are expressed as stimulation index (SI) corresponding to cpm in cultures with peptide/cpm in cultures without peptide. A mean SI $\geq$3.0 in the proliferation test was considered as positive. The tritiated thymidine incorporation in the absence of peptide was comprised between 100 and 2000 cpm. *B*, PBMCs from SLE patients were stimulated ex vivo in the presence of increasing concentrations of peptides 131–151, Sc, P140, and ScP, and proliferation was measured after 7 days of culture. Results are expressed as SI. The results from four representative patients are shown in the figure.

To confirm the T cell proliferation data and demonstrate that recognition of peptide 131–151 was restricted to $CD4^+$ T cells, PBMCs from three peptide 131–151-responder patients were stained with the viable fluorescent dye CFSE to allow visualization of the cell expansion. Cells were cultured in the presence or absence of peptide 131–151, collected and stained with anti-CD4 mAbs labeled with allophycocyanin. Forward and side scatter analysis revealed that cells cultured in the presence of peptide 131–151 migrated into the "blast" light scatter population (Fig. 2*A*). Plots were then gated by light scatter to include all viable T lymphocytes (i.e., resting and blast cells corresponding to R1) and a two-color analysis of cells were used to distinguish CD4-positive and CD4-negative populations (R2 and R3, respectively). Staining of CFSE-labeled cells with anti-CD4 mAb showed an increased number of $CD4^+$ T cells in the later generation in response to

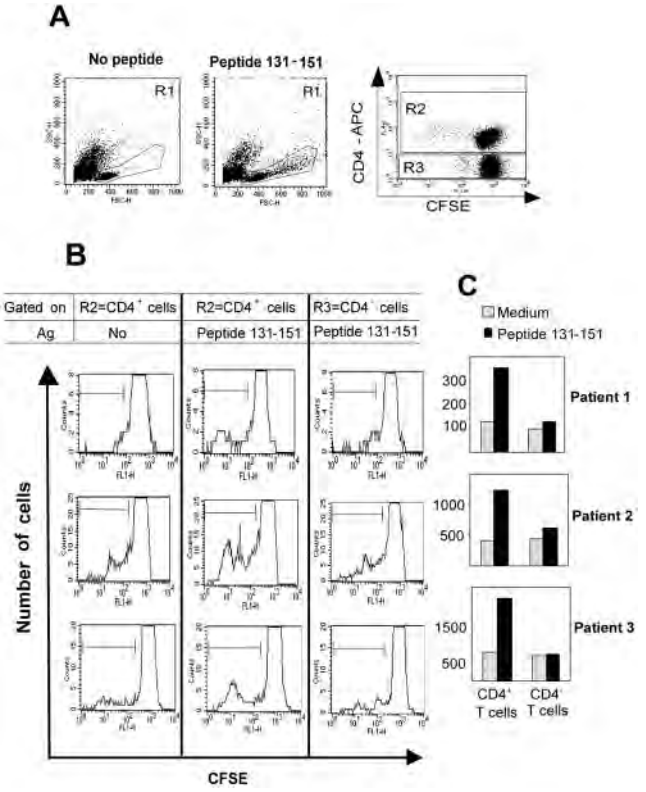

FIGURE 2. Proliferation of PBMCs from SLE patients in response to peptide 131–151 is mediated by $CD4^+$ T cells. *A*, Lymphocytes from a peptide 131–151-responder patient shown as example cultured in the presence or absence of peptide 131–151 were gated in a forward vs side scatter dot plot (R1). Two fluorescent analyses (CFSE vs $CD4^-$ allophycocyanin) were used to established gates R2 and R3 corresponding to CD4-positive cells or CD4-negative cells, respectively. *B*, Histograms gated on R2 or R3 were used to determine the extent of progressive reduction in CFSE intensity related to cell division when cells were cultured in the presence or absence of peptide 131–151. *C*, Summary of data obtained from dot blot analysis. The numbers of cells that proliferated in response to peptide 131–151 was determined by calculating the number of cells showing a reduction in CFSE intensity ($CFSE^{dim}$) based on a fixed number (5000) of $CFSE^{bright}$ cells. The numbers of $CD4^+$ T cells that proliferated in response to Con A are 2690, 3762, and 4333 for patients 1, 2, and 3, respectively.

peptide 131–151 (Fig. 2*B*, *left* and *middle panels*) but not when cells were cultured with the control peptide Sc (data not shown). No significant reduction in CFSE intensity was observed in response to peptide 131–151 in the $CD4^-$ population (Fig. 2*B*, *right*). Combined data from these experiments, expressed as numbers of $CFSE^{dim}$ cells (Fig. 2*C*) showed that $CD4^+$ T cells are the major population proliferating in the presence of peptide 131–151. In the same CFSE test with PBMCs from the same and other lupus patients, P140 peptide did not induce cell division. Taken together, these data demonstrate that peptide 131–151 very specifically stimulates peripheral lupus $CD4^+$ T cells, and that in contrast to $CD4^+$ T cells from lupus mice, P140 peptide is unable to stimulate division and proliferation of patients $CD4^+$ T cells.

Binding of peptides 131–151 and P140 to HLA-DR class II molecules

The apparent lack of effect of the P140 analog with PBMCs and CFSE-labeled $CD4^+$ T cells from lupus patients was intriguing. This result contrasted with our previous data showing that in MRL/*lpr* mice peptide 131–151 and its analog P140 were recognized equally well by lupus T cells (10). Moreover, in an in vitro assay, we also demonstrated previously that both peptides bound to the three HLA class II molecules studied, namely DR1, DR4, and DR11 (10). Using another binding test based on HLA molecules affinity-purified from EBV homozygous cell lines (16–18), we screened a more complete set of class II HLA-DR molecules and confirmed that both peptides 131–151 and P140 effectively bound several DR molecules (Table II). In this assay, both peptides 131–151 and P140 did not bind DRB3 and DRB4. However, they bound to DR1, DR3, DR4, DR7, DR11, DR15 (the B chain of DR2), and DRB5 molecules with "good" or "intermediary" affinity (as determined in the calibration test), and peptide 131–151 also bound to DR13 with a "good" affinity (Table II). The results thus suggest that both peptides have the capacity to bind most of the tested DR molecules.

Modeling of peptides 131–151 and P140 within HLA-DR molecules

Using the known x-ray structures of MHC class II molecules DR1, DR2, DR3, and DR4 associated with peptides, we then modeled the peptides 131–151 and P140 into the binding groove of these four DR molecules (Fig. 3; Table III). Because the structures of DR11, DR13, and DRB5-peptide complexes are not known, we extrapolated the possible interactions of peptides 131–151 and P140 into DR11 and DR13 from the DR3 structure that is very close, and into DRB5 from that of DR1 that is very close to DRB5. In good agreement with the results described in Table II with purified DR molecules, in the case of DR1 and DR4, the peptides 131–151 and P140 showed a relatively low binding capacity due to

Table II. *Capacity of U1-70K protein sequence 131–151 and of its phosphorylated analog to bind to HLA-DR molecules*[a]

	DR1	DR3	DR4	DR7	DR11	DR13	DR15	DRB3	DRB4	DRB5
131–151	650	1,000	6,000	625	95	3,333	667	>10,000	>10,000	90
	(**320**)	(**3.5**)	(**200**)	(**60**)	(**5**)	(**7**)	(**45**)			(**9**)
P140	550	60,000	10,000	3,375	350	>100,000	450	>10,000	>10,000	125
	(**270**)	(**210**)	(**330**)	(**340**)	(**18**)		(**30**)			(**12**)
Sc	7,500	200	>100,000	60,000	33	850	800	>10,000	>10,000	2,750
	3,750	(**0.7**)		6,000	(**1.6**)	(**1.8**)	(**55**)			(**275**)
ScP	1,750	140	25,000	7,500	12	250	500	>10,000	>10,000	650
	(**870**)	(**0.5**)	(**830**)	(**750**)	(**0.6**)	(**0.5**)	(**35**)			(**65**)

[a] Quantity of peptide bound to HLA-DR molecules was evaluated in a fluorescent solid-phase assay. Data are expressed as the peptide amount in nanomoles that prevented binding of 50% of the biotinylated peptide (IC_{50}). Maximal binding was determined by incubating the biotinylated peptide with the purified HLA class II molecules in the absence of cold competitor. Average values were deduced from at least two independent experiments. Unlabeled forms of the biotinylated peptides were used as reference peptides to assess the validity of each experiment. Their IC_{50} variation did not exceed a factor of 3. Average values of the reference biotinylated peptides were the following: HA306–318 (DR1, 2 nM; DR4, 30 nM; DR11, 20 nM; DRB5, 10 nM), MT 2–16 (DR3, 280 nM), YKL (DR7, 10 nM), B21–36 (DRB*1301, 470 nM), A3 152–166 (DRB1*1501, 15 nM), LOL 191–210 (DRB3*0101, 15 nM), and E2/E168 (DRB4*0101; 10 nM). Numbers in parentheses reflect the ratio between the IC_{50} of the competitor peptide and that of the reference peptide. Values lower than 20 (in bold and underlined) indicate that the peptide binds to HLA-DR molecules with a "good" affinity. Values comprised between 20 and 1,000 (in bold) indicate that the peptide binds to HLA-DR molecules with an "intermediary" affinity. The values >1,000 correspond to nonbinders.

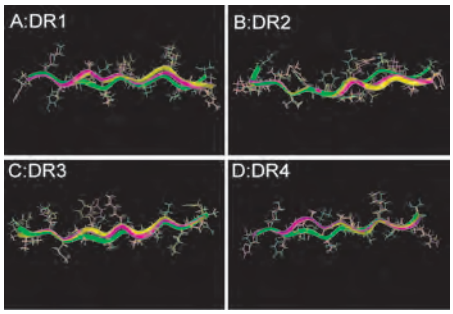

FIGURE 3. Conformations of peptides 131–151 and P140 in the binding sites of four HLA class II DR molecules as deduced by molecular modeling from the respective HLA-peptide crystal structures. The structure of the cognate peptides (in green), peptide 131–151 (in magenta) and P140 (in yellow) are shown into the DR binding groove (DRB1*0101; *A*), DRB1*1501 (*B*), DRB1*030101 (*C*), and DRB1*0401 (*D*) HLA molecules. The N terminus of peptides is at the *left*, the C terminus at the *right*. HLA anchoring residues are shown directed to the bottom, potential TCR contact residues are shown pointing to the top. The backbone is emphasized as a solid oval ribbon. Details of the structures are summarized in Table II.

a significant shifting of the peptide backbones in the groove, leading to the loss of three to four hydrogen bonds that are important for stabilizing the peptides into the DR molecules (Fig. 3, *A* and *D*).

In the case of the DR2 molecule, while the peptides 131–151 and P140 also undergo a significant shift (Fig. 3*B*), this shift is clearly restricted to the C-terminal end of the peptide, which has been shown in the case of crystallized peptide within DR2 to be less well-anchored into the binding groove (20). Almost all the hydrogen bonds established between the peptide and residues of the DR2 molecule are limited to the N terminus of the peptide and are completely conserved in peptides 131–151 and P140. This model thus supports the results found with purified DR molecules (Table II) showing a good binding capacity of both peptides to the DR15 molecule. Modeling the peptide 131–151 in the DR3 molecule only resulted in a small shift of the peptide into the groove, allowing the conservation of 12 of 14 hydrogen bonds between the peptide backbone and the DR3 molecule (Fig. 3*C*; Table III). The anchoring residues correspond to a second motif with Ser in pocket 4 and Arg in pocket 6 (21). The peptide 131–151 thus possesses a good binding capacity for DR3. The relatively lower binding capacity of the analog P140 found in the in vitro assay described in Table II could be due to increased difficulty of P140 to accommodate the small cavity of pocket 7 limited by Arg B74 and Gln^{B70}. In view of the high similarity between DR1 and DRB5 sequences and the difference in their capacity to bind the peptide 131–151 and the analog P140 (factor 20–40; Table II), a model of DRB5 molecule was built starting from the DR1 molecule. Interestingly, peptide 131–151 was able to bind to DRB5 using Ile^{132} as anchoring residue in pocket 1 and Val^{135} in pocket 4. The mutations from Glu^{B28} to His^{B28} and Leu^{B67} to Phe^{B67} between DR1 and DRB5 close pocket 7 in DRB5 obliging the Lys^{138} of peptide to point toward the surface. Modeling the peptide in DR1 using the same anchoring residues resulted in an energetically unfavorable interaction of Lys^{138} with pocket 7, and therefore this model was not retained.

Interestingly, DR13 was found in the binding assay to represent the only DR molecule with a high selectivity for the nonphosphorylated peptide 131–151 over the phosphorylated analog P140 (Table II). We modeled both peptides within the DR13 molecule, which possesses a single change compared with DR3 in the binding groove. In complete agreement with the data reported in Table II, we observed that the mutation of Lys^{B71} in DR3 to Glu^{B71} in DR13 acidifies pocket 7 thus prohibiting the anchoring of the negatively charged phosphoserine 140 in the pocket.

In a similar manner, we modeled the peptides within DR11 which is very similar to DR3. We observed that DR11 could effectively anchor the phosphoserine residue 140 because Arg^{B74} and Gln^{B70}, which limit the interaction of peptide P140 into DR3, have been mutated to Ala^{B74} and Asp^{B70}, respectively, in DR11. These results thus completely support the data obtained in the in vitro binding assay (Table II).

Immunogenetic association of SLE patients' HLA with the ability of their PBMCs to proliferate with peptide 131–151

We determined the genotypes of HLA class I and II alleles of all SLE patients included in this study and listed their allelic frequencies among the patients with or without peptide 131–151-proliferating PBMCs (Table IV). As it could be expected from the above results, no specific association between patients' HLA haplotypes and cellular responses to peptide 131–151 was observed. Remarkably, all peptide 131–151 responders possessed at least one of the DR molecules (in bold in Table IV) shown to associate with peptide 131–151, namely DR1, DR3, DR4, DR7, DR11, DR13, DR15, and DRB5.

PBMCs from patients with SLE do not proliferate ex vivo in the presence of peptide analog P140 but secrete high levels of IL-10

We have shown above that a significant proportion of lupus patients had T cells reacting with peptide 131–151, that this reactivity seemed to be associated to a large set of HLA-DR molecules, and that while the analog P140 also associated to the majority of these HLA-DR molecules, it did not induce ex vivo any proliferation of PBMCs from these patients. This result could reveal an absence of reactivity of patients' T cells with the analog P140 due to an alteration of its recognition by the TCR resulting from the presence of the phosphoserine in position 140. Alternatively, the observed lack of reactivity of PBMCs in the presence of peptide P140 could reflect a particular property of analog P140 that for example behaves as an APL of the TCR (23, 24). In the latter hypothesis, P140 presented in the context of MHC molecules might interact with elements of the TCR, but instead of inducing the classical cascade of events required for full T cell activation, it might affect the normal T cell signaling pathway and classical pattern of cytokine secretion, and consequently alter T cell activation. To address these issues, examining the activation markers, such as CD69, CD25, or CD79, at the surface of T cells was not reliable since it is known that in lupus patients, autoimmune T cells are preactivated in vivo (4). We thus examined the newly synthesized cytokine pattern of PBMCs stimulated ex vivo by both peptides 131–151 and P140. We screened fresh PBMCs from a second series of patients corresponding to 16 unselected, consecutive patients with SLE (four patients, namely Bou, Bri, Peu and Tua, were common in series 1 and 2) and 14 control individuals including 4 healthy individuals, 4 patients hospitalized for nonautoimmune diseases, and 6 patients with rheumatoid arthritis and/or primary Sjögren's syndrome. We confirmed with this second series of lupus patients that analog P140 was unable to induce a proliferative T cell response ex vivo. No significant levels of IFN-γ and IL-4 were measurable in the cultures. However, as shown in Fig. 4*A*, PBMCs from 8 of 16 patients with SLE (50%) secreted large amounts of IL-10 in response to peptide P140, in average 15-fold higher than the background (45-fold in one patient; Fig. 4*A*). This result is highly significant because it demonstrates that T cells from these patients recognize the P140 peptide analog. Low levels of IL-10

Table III. *Interaction of peptide 131–151 with the DR molecules as deduced from molecular modeling*

	R	I	H	M	V	Y	S	K	R	S	G	K	P	R	G	Y	A	F	I	E	Y		
	Anchoring residues																					H-Bonds	RMS (Å)
DR1					1			4		6			9									8/12	0.43
DR2					1			4		6			9									8/9	0.52
DR3				1			4		6			9										12/14	0.30
DR4					1			4		6			9									9/14	0.40
DRB5	1				4		6			9												11/13	0.26

[a] H-Bonds correspond to hydrogen bonds between the peptide backbone and the DR molecule. RMS is the deviation in Å between the Cα of the 131–151 peptide compared to the cognate peptide crystallized in the DR molecule structure. For DRB5, the DR1 model was used.

secretion in response to peptide P140 were found in one patient only of the control group (ratio of 3.2 at the limit of positivity in the case of patient Ric suffering from pSS; $p = 0.009$). IL-10 secretion by PBMCs of SLE patients was dose-dependent (shown in Fig. 4*B* with four representative examples) and was blocked by the addition of mAbs directed to HLA class-II molecules (DP, DQ, and DR, not shown), indicating that the major population of cells secreting IL-10 in the PBMC fraction corresponds to $CD4^+$ T cells. It was peptide P140-specific as demonstrated with the phosphorylated scrambled peptide ScP that induced much lower levels of IL-10 in the cultures (Fig. 4*A*). As compared with IL-10 secretion levels induced by peptide P140, those induced by the 131–151 peptide tested in parallel were equivalent or lower (Fig. 4*B*). We found no significant correlation between lupus patients who were P140 responders and any of their clinical or biological signs. However, as above, it is noteworthy that among the eight positive patients (eight females), four (Bon, Mah, Sie, Tua) had no anti-dsDNA Abs at the time of the study and four (Bog, Bon, Sie, Tua) had no ENA. All P140 responders possessed at least one of the DR molecules (in bold) shown to associate with peptide P140, namely DR1, DR3, DR4, DR7, DR11, DR15, and DRB5 (Table V).

Discussion

We have explored the spontaneous responsiveness of PBMCs from lupus and nonlupus autoimmune individuals to peptide 131–151 and its phosphorylated peptide analog P140. As expected from our previous studies with murine MHC molecules (8–10), we found that both peptides bind to a large panel of HLA class II molecules. This was confirmed in two distinct in vitro assays (Ref. 10, and the present study) and is supported further here by molecular modeling experiments performed in silico. Remarkably, both peptides 131–151 and P140 undergo significant shifts within the binding groove of different HLA molecules, and for each molecule distinct anchor residues of peptides accommodate residues in different MHC pockets generating thus different peptide-MHC surfaces for TCR interaction. We found no association between HLA-DR/DQ/DP phenotypes of our two cohorts of SLE patients and the capacity of PBMCs of these patients to proliferate and secrete IL-10 in the presence of peptides 131–151 and P140.

PBMCs from about half of the SLE patients examined recognize the peptide 131–151 and its phosphorylated analog P140. However, in contrast to our previous findings in unprimed lupus mice, the parent peptide only and not the P140 analog, stimulated ex vivo proliferation of PBMCs. Both generated IL-10 secretion in the cultures. Thus, we show here a new and unexpected property of the P140 analog, which in the presence of lupus patients' PBMCs fails to induce proliferation and induces a strong IL-10 response. Based on these results, it seems therefore that in the specific stage of activation of T cells collected from lupus patients, peptide P140 might behave not only as a promiscuous epitope but also as an APL capable of altering proliferation of T cells and up-regulating the production of anti-inflammatory cytokine IL-10. If this hypothesis is true, our results indicate that the P140 analog could rather act as a partial agonist of the TCR that only affects a subset of T cell signaling response, and not as an antagonist, which leads to total anergy to T cells (23, 24). It has been shown that APLs can polarize the differentiation of uncommitted Th cells (Th0), resulting in individual T cell subsets that only secrete specific cytokines by an immune deviation process. Apart from IL-10, this feature was not observed in our study in the limit of cytokine panel tested (i.e., IL-2, IL-4, and IFN-γ). It is possible, however, that other Th2 type of cytokines such as IL-5 or TGF-β are up-regulated while proinflammatory cytokines (e.g., TNF-α, TNF-β that were not tested here) are down-regulated, contributing thus to the immune deviation generated by the P140 analog.

Another functional hypothesis could also be envisaged. The scheme depicted above, namely the capacity of peptide P140 to induce high secretion levels of IL-10 (and no IL-4 or IFN-γ) in the absence of any PBMC proliferation might also be explained by the fact that peptide P140 specifically activates a subset of regulatory T cells. This cell population includes naturally arising $CD4^+CD25^+$ regulatory T cells (25), the number of which is decreased in peripheral blood during active lupus disease (26, 27), and adaptive regulatory T cells induced in response to specific tolerogenic stimuli. The latter correspond to Tr1 regulatory T cells producing IL-10 (28) and Th3 cells that confer immunosuppressive effects by TGF-β secretion (29). A mapping study aimed at identifying Th epitopes of the major RBC autoantigen in patients with autoimmune hemolytic anemia revealed that particular peptides of this Ag selectively stimulated Tr1 cells by eliciting IL-10 secretion in the absence of proliferation (30). Interestingly, as in the case of peptide P140, certain peptides stimulate IL-10 production in most of the autoimmune hemolytic anemia patients, despite variation of HLA haplotypes, suggesting that they are presented promiscuously by a variety of HLA class II molecules. It is obviously tempting to compare these recent studies to the findings described here with the analog P140, and to speculate that peptide P140 might have the ability to stimulate specific regulatory T cells.

The balance between Th1 cells (secreting IL-2, IFN-γ, and/or lymphotoxin-α) and Th2 cells (usually classified as secreting IL-4, IL5, IL-10, and TGF-β) is central in SLE and many other autoimmune diseases. Recent studies in lupus have specifically addressed the relative contribution of IL-10, which seems to be secreted in mass when patients' PBMCs are incubated in the presence of peptide analog P140. This cytokine is generally regarded first as an immunosuppressive cytokine but its biology is complex and it exhibits pleiotropic pro- and anti-inflammatory effects (31, 32). Although s.c. administrations of IL-10 have been shown to induce modest but significant improvements in clinical

Table IV. *HLA class I and II alleles of the first series of SLE patients and T cell response to peptide 131–151 of the U1-70K protein*

Patients (n = 22)	HLA Typing Class I Molecules	Class II Molecules	T Cell Response to Peptide 131–151
Ban	A1 A32 B8 B63 (15)	DRB1***01*03** DQB1*05*02	+
Ben	A1 A2 B35 B51 (5)	DRB1*14***07** DQB1*05*02	−
Blo	A2 B7 B57 (17)	DRB1***04*04** DQB1*03*03	+
Bou	A2 B62 (15) B50 (21)	DRB1***07** DQB1*02*03	+
Bri	A2 A24(9) B44(12) B60(40)	DRB1***07*15** DQB1*02*06	−
Cha	A2 B7 B45 (12)	DRB1***15*13** DQB1*06	+
Did	A1 A3 B8 B27	DRB1*16***03** DQB1*05*02	−
Dol	A2 B44(12) B51(5)	DRB1***04** DQB1*03*03	−
Fil	A3 A25 (10) B8 B44 (12)	DRB1***03***14 DQB1*02*05	+
Gio	A2 A29(19) B35 B49(21)	DRB1***11** DQB1*03	−
Gru	A*01*03 B*08*50	DRB1***15*07** DQB1*06*02	−
Izo	A1 A3 B8 B18	DRB1***04*13** DQB1*03*06	+
Iac	A*02 B7 B44 (12)	DRB1***04*15** DQB1*06*03	−
Laz	A1 A3 B7 B8	DRB1***15*03** DQB1*06*02	−
Le	A2 A33 (19) B51 (5) B60 (40)	DRB1***15***09 DQB1*06*03	−
Pel	A1 A25 (10) B8 B62 (15)	DRB1***03*11** DQB1*02*03	+
Peu	A11 A33 (19) B13 B58 (17)	DRB1*16***03** DQB1*05*02	−
Piq	A2 A10 B8 B70	DRB1***15***09 DQB1*06*02	−
Sch	A3 A68(28) B7 B44(12)	DRB1***01*15** DQB1*05*06	−
Scho	A1 A23(9) B8 B35	DRB1***03*13** DQB1*02*06	−
Tua	A2 A28 B35 B8	DRB1***03*04** DQB1*02*03	+
Woz	A1 A3 B7 B8	DRB1***15*03** DQB1*06*02	−

[a] HLA class II alleles in bold are those that have been demonstrated to interact with peptide 131–151 in in vitro assays.

trials including patients with rheumatoid arthritis, Crohn's disease, and psoriasis, for example, in SLE the effect was apparently the opposite, and anti-IL-10 strategies have been proposed instead to reduce the disease (33). In the MRL/*lpr* lupus model, however, IL-10 has been shown to regulate the lupus disease through the inhibition of pathogenic Th1 response (34). In BW mice the induction of T cells producing IL-10 has been associated in vitro with a reduction of anti-dsDNA Abs (35). Thus, this question remains completely open and it is possible that depending on the tissue, the target cells (Th1 cells, NK cells, endothelial cells, macrophages, mast cells, or activated/resting B cells) and the stage/duration of the disease, distinct effects appear on the inflammatory state.

At this stage, however, several key questions have to be addressed regarding the capacity of the P140 analog to form stable or short-lived complexes with HLA molecules (36), and once it is recognized in this context by TCRs to alter signaling and functions of autoreactive Th clones. An important issue will be to demonstrate that this does not modify the normal behavior of other Th or CTLs. Further characterization of regulatory T cell subsets possibly involved in this process will be determining. Defining whether a restricted or a broad family of TCRs is implicated in this mechanism will be also highly informative, because apparently there is an important difference between murine and human lupus with regard to the recognition of peptide P140 by $CD4^+$ T cells. It will be interesting to compare the data with those obtained with U1-70K protein-reactive human T cell clones, the TCR CDR3 of which have been sequenced and found to be highly conserved (37).

Several peptide candidates acting as APLs of TCRs of autoimmune Th cells have been described in autoimmune diseases and are being evaluated in clinical trials (38–40). Immune deviation by TCR antagonism seems to have the potential to be used therapeutically to significantly delay undesired T cell responses that ultimately lead to disease (41). Alternatively, peptides able to stimulate regulatory T cells might also represent valuable candidates for deviating a proinflammatory autoimmune pattern to a more regulatory functional phenotype. These two possible mechanisms,

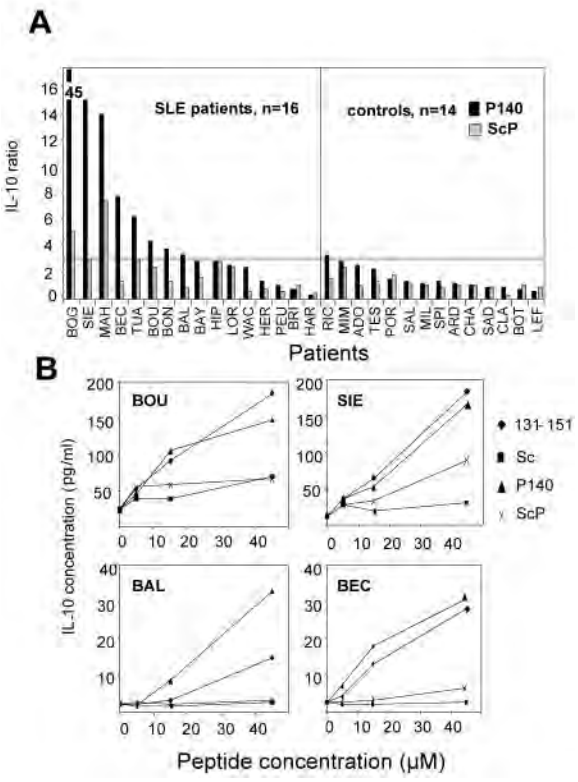

FIGURE 4. IL-10 secretion of SLE PBMCs in response to phosphorylated peptide P140. *A*, PBMCs from 16 patients with SLE and 14 controls were cultured in the presence of 15 μM of peptides P140 (■) and ScP (▨). Supernatants were collected 24 h later, and IL-10 production was tested by ELISA. Results are expressed as IL-10 ratio, corresponding to IL-10 concentration in the presence of peptide/IL-10 concentration in the absence of peptide. A mean ratio $\geq$3.0 is considered as positive. *B*, PBMCs from SLE patients were stimulated ex vivo in the presence of increasing concentrations of peptides 131–151, Sc, P140, and ScP, and IL-10 secretion was measured after 24 h. The results from four representative patients are shown in the figure.

Table V. *HLA class I and II alleles of the second series of SLE patients and T cell response to the P140 analog (IL-10 secretion) of the U1-70K protein*[a]

Patients (n = 16)	HLA Typing Class I Molecules	Class II Molecules	T Cell Response to Peptide P140
Bal	A2 A3 B7 B57 (17)	DRB1***15*11** DQB1*06*03	+
Bay	A2 A29 (19) B45(12) B51(5)	DRB1***07***13 DQB1*02*06	−
Bec	A3 A30(19) B18 B27	DRB1***03*04** DQB1*02*03	+
Bog	A*33*68 B*14*39	DRB1***01***16 DQB1*05	+
Bon	A*24(9)*26(10) B*07*55	DRB1***15** DQB1*06	+
Bou	A2 B62 (15) B50 (21)	DRB1***07** DQB1*02*03	+
Bri	A2 A24(9) B44(12) B60(40)	DRB1***07*15** DQB1*02*06	−
Har	A*01*02 B*08*44	DRB1***15*04** DQB1*06*03	−
Her	A03 B07	DRB1***15** DQB1*06	−
Hip	A2 A24(9) B51 (5) B55 (22)	DRB1*16***04** DQB1*05*03	−
Lor	A23 (9) A24 (9) B8	DRB1***15*03** DQB1*06*02	−
Mah	A1 A2 B*51*53	DRB1*13***15** DQB1*05*06	+
Peu	A11 A33 (19) B13 B58 (17)	DRB1*16***03** DQB1*05*02	−
Sie	A2 A32 B7 B44	DRB1***04***16 DQB1*03*05	+
Tua	A2 A28 B35 B8	DRB1***03*04** DQB1*02*03	+
Wac	A3 A24 (9) B7 B63 (15)	DRB1***15*04** DQB1*06*03	−

[a] HLA class II alleles in bold are those that have been demonstrated to interact with peptide P140 in in vitro assays.

not necessarily independent, can theoretically be used by our candidate analog P140. Dissecting these pathways should help us to better understand how to promote a robust regulatory state in lupus for reversing the disease progression.

Acknowledgments

We thank A. Dormoy (Blood Center, Strasbourg, France) for HLA typing and J.-C. Peter (CNRS, Strasbourg, France) for statistical analysis of results.

Disclosures

The authors have no financial conflict of interest.

References

1. Murata, H., R. Matsumura, A. Koyama, T. Sugiyama, M. Sueishi, K. Shibuya, A. Tsutsumi, and T. Sumida. 2002. T cell receptor repertoire of T cells in the kidneys of patients with lupus nephritis. *Arthritis Rheum.* 46: 2141–2147.
2. Talken, B. L., K. R. Schäfermeyer, C. W. Bailey, D. R. Lee, and R. W. Hoffman. 2001. T cell epitope mapping of the Smith antigen reveals that highly conserved Smith antigen motifs are the dominant target of the T cell immunity in systemic lupus erythematosus. *J. Immunol.* 167: 562–568.
3. Okubo, M., M. Kokubun, T. Nishimaki, R. Kasukawa, H. Ohto, K. Yamamoto, and S. Muller. 1995. T cell epitope mapping of U1-A RNP. *Arthritis Rheum.* 38: 1170–1172.
4. Lu, L., A. Kaliyaperumal, D. T. Boumpas, and S. K. Datta. 1999. Major peptide autoepitopes for nucleosome-specific T cells of human lupus. *J. Clin. Invest.* 104: 345–355.
5. Greidinger, E. L., M. F. Foecking, K. M. Schäfermeyer, C. W. Bailey, S. L. Primm, D. R. Lee, and R. W. Hoffman. 2002. T cell immunity in connective tissue disease patients targets the RNA binding domain of the U1–70 kDa small nuclear ribonucleoprotein. *J. Immunol.* 169: 3429–3437.
6. Riemekasten, G., C. Weiss, S. Schneider, A. Thiel, A. Bruns, F. Schumann, S. Bläss, G. R. Burmester, and F. Hiepe. 2002. T cell reactivity against the SmD1(83–119) C-terminal peptide in patients with systemic lupus erythematosus. *Ann. Rheum. Dis.* 61: 779–785.
7. Davies, M. L., E. J. Taylor, C. Gordon, S. P. Young, K. Welsh, M. Bunce, B. P. Wordsworth, B. Davidson, and S. J. Bowman. 2002. Candidate T cell epitopes of the human La/SSB autoantigen. *Arthritis Rheum.* 46: 209–214.
8. Monneaux, F., J.-P. Briand, and S. Muller. 2000. B and T cell immune response to snRNP in lupus mice: autoreactive $CD4^+$ T cells recognize a T cell epitope located within the conserved RNP consensus sequence of the 70K protein. *Eur. J. Immunol.* 20: 2191–2200.
9. Monneaux, F., H. Dumortier, G. Steiner, J.-P. Briand, and S. Muller. 2001. Murine models of systemic lupus erythematosus: B and T cell responses to spliceosomal ribonucleoproteins in MRL/Fas *lpr* and $(NZB \times NZW)F_1$ lupus mice. *Int. Immunol.* 13: 1155–1163.
10. Monneaux, F., J. M. Lozano, M. E. Patarroyo, J.-P. Briand, and S. Muller. 2003. T cell recognition and therapeutic effect of a phosphorylated synthetic peptide of the 70K snRNP protein administered in MRL/*lpr* mice. *Eur. J. Immunol.* 33: 287–296.
11. Monneaux, F., V. Parietti, J.-P. Briand, and S. Muller. 2004. Intramolecular T cell spreading in unprimed MRL/*lpr* mice: importance of the U1–70K protein sequence 131–151. *Arthritis Rheum.* 50: 3232–3238.
12. Monneaux, F., and S. Muller. 2001. Key sequences involved in the spreading of the systemic autoimmune response to spliceosomal proteins. *Scand. J. Immunol.* 54: 45–54.
13. Monneaux, F., and S. Muller. 2002. Epitope spreading in systemic lupus erythematosus: identification of triggering peptide sequences. *Arthritis Rheum.* 46: 1430–1438.
14. Kaliyaperumal, A., M. A. Michaels, and S. K. Datta. 1999. Antigen-specific therapy of murine lupus nephritis using nucleosomal peptides: tolerance spreading impairs pathogenic function of autoimmune T and B cells. *J. Immunol.* 162: 5775–5775.
15. Bombardier, C., D. D. Gladman, M. B. Urowitz, D. Caron, C. H. Chang, and the Comittee on Prognosis Studies in SLE. 1992. Derivation of the SLEDAI: a disease activity index for lupus patients. *Arthritis Rheum.* 35: 630–640.
16. Fournel, S., S. Neichel, H. Dali, S. Farci, B. Maillère, J.-P. Briand, and S. Muller. 2003. $CD4^+$ T cells from $(NZB \times NZW)F_1$ lupus mice and normal mice immunized against apoptotic nucleosomes recognize similar Th cell epitopes in the C terminus of histone H3. *J. Immunol.* 171: 636–644.
17. Texier, C., S. Pouvelle, M. Busson, M. Hervé, D. Charron, A. Ménèz, and B. Maillère. 2000. HLA-DR restricted peptide candidates for bee venom immunotherapy. *J. Immunol.* 164: 3177–3184.
18. Texier, C., S. Pouvelle-Moratille, M. Busson, D. Charron, A. Ménèz, and B. Maillère. 2001. Complementarity and redundancy of the binding specificity of HLA-DRB1, -DRB3, -DRB4 and -DRB5 molecules. *Eur. J. Immunol.* 31: 1837–1846.
19. Hennecke, J., A. Carfi, and D. C. Wiley. 2000. Structure of a covalently stabilized complex of a human $\alpha\beta$ T-cell receptor, influenza HA peptide and MHC class II molecule, HLA-DR1. *EMBO J.* 19: 5611–5624.
20. Smith, K. J., J. Pyrdol, L. Gauthier, D. C. Wiley, and K. W. Wucherpfennig. 1998. Crystal structure of HLA-DR2 (DRA*0101, DRB1*1501) complexed with a peptide from human myelin basic protein. *J. Exp. Med.* 188: 1511–1520.
21. Ghosh, P., M. Amaya, E. Mellins, and D. C. Wiley. 1995. The structure of an intermediate in class II MHC maturation: CLIP bound to HLA-DR3. *Nature* 378: 457–462.
22. Hennecke, J., and D. C. Wiley. 2002. Structure of a complex of the human α/β T cell receptor (TCR) HA1.7, influenza hemagglutinin peptide, and major histocompatibility complex class II molecule, HLA-DR4 (DRA*0101 and DRB1*0401): insight into TCR cross-restriction and alloreactivity. *J. Exp. Med.* 195: 571–581.
23. Sloan-Lancaster, J., and P. Allen. 1996. Altered peptide ligand-induced partial T cell activation: molecular mechanisms and role in T cell biology. *Annu. Rev. Immunol.* 14: 1–27.
24. Nicholson, L., and V. Kuchroo. 1997. T cell recognition of self and altered self antigens. *Crit. Rev. Immunol.* 17: 449–462.
25. Sakaguchi, S., N. Sakaguchi, M. Asano, M. Itoh, and M. Toda. 1995. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor α-chains (CD25): breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J. Immunol.* 155: 1151–1164.
26. Crispin, J. C., A. Martinez, and J. Alcocer-Varela. 2003. Quantification of regulatory T cells in patients with systemic lupus erythematosus. *J. Autoimmun.* 21: 273–276.
27. Liu, M.-F., C.-R. Wang, L.-L. Fung, and C.-R. Wu. 2004. Decreased $CD4^+CD25^+$ T cells in peripheral blood of patients with systemic lupus erythematosus. *Scand. J. Immunol.* 59: 198–202.
28. Groux, H., A. O'Garra, M. Bigler, M. Rouleau, S. Antonenko, J. E. de Vries, and M. G. Roncarolo. 1997. A $CD4^+$ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. *Nature* 389: 737–742.

29. Chen, Y., V. K. Kuchroo, J. Inobe, D. A. Hafler, and H. L. Weiner. 1994. Regulatory T cell clones induced by oral tolerance: suppression of autoimmune encephalomyelitis. *Science* 265: 1237–1240.
30. Hall, A. M., F. J. Ward, M. A. Vickers, L. M. Stott, S. J. Urbaniak, and R. N. Barker. 2002. Interleukin-10-mediated regulatory T-cell responses to epitopes on a human red blood cell autoantigen. *Blood* 100: 4529–4536.
31. Mocellin, S., M. C. Panelli, E. Wang, D. Nagorsen, and F. M. Marincolla. 2003. The dual role of IL-10. *Trends Immunol.* 24: 36–43.
32. Conti, P., D. Kempuraj, K. Kandere, M. Di Gioacchino, R. C. Barbacane, M. L. Castellani, M. Felaco, W. Boucher, R. Letourneau, and T. C. Theoharides. 2003. IL-10, an inflammatory/inhibitory cytokine, but not always. *Immunol. Lett.* 86: 123–129.
33. Llorente, L., Y. Richaud-Patin, C. Garcia-Padilla, E. Claret, J. Jakez-Ocampo, M. H. Cardiel, J. Alcocer-Varela, L. Grangeot-Keros, D. Alarcon-Segovia, J. Wijdenes, et al. 2000. Clinical and biologic effects of anti-IL-10 monoclonal antibody administration in systemic lupus erythematosus. *Arthritis Rheum.* 43: 1790–1800.
34. Yin, Z., G. Bahtiyar, N. Zhang, L. Liu, P. Zhu, M. E. Robert, J. McNiff, M. P. Madaio, and J. Craft. 2002. IL-10 regulates murine lupus. *J. Immunol.* 169: 2148–2155.
35. Riemekasten, G., D. Langnikel, P. Enghard, R. Undeutsh, J. Humrich, F. M. Ebling, B. Hocher, T. Humaljoki, H. Neumayer, G. R. Burmester, et al. 2004. Immune tolerance to the $SmD1_{83–119}$ peptide postpones development of murine lupus and induces regulatory Tr1 cells. *J. Immunol.* 173: 5835–5842.
36. Mirshahidi, S., L. C. Korb Ferris, and S. Sadegh-Nasseri. 2004. The magnitude of TCR engagement is a critical predictor of T cell anergy or activation. *J. Immunol.* 172: 5346–5355.
37. Talken, B. L., C. W. Bailey, S. L. Reardon, C. W. Caldwell, and R. W. Hoffman. 2001. Structural analysis of TCRα and β chains from human T-cell clones specific for small nuclear ribonucleoprotein polypeptides Sm-D, Sm-B and U1–70K: TCR complementary determining region 3 usage appears highly conserved. *Scand. J. Immunol.* 54: 204–210.
38. Kappos, L., G. Comi, H. Panitch, J. Oger, J. Antel, P. Conlon, and L. Steinman. 2000. Induction of a non-encephalitogenic type 2 T helper-cell autoimmune response in multiple sclerosis after administration of an altered peptide ligand in a placebo-controlled, randomized phase II trial. *Nat. Med.* 6: 1176–1182.
39. Masteller, E. L., and J. A. Bluestone. 2002. Immunotherapy of insulin-dependent diabetes mellitus. *Curr. Opin. Immunol.* 14: 652–659.
40. Luger, D., M. Dayan, H. Zinger, J.-P. Liu, and E. Mozes. 2004. A peptide based on the complementarity region 1 of a human monoclonal autoantibody ameliorates spontaneous and induced lupus manifestations in correlation with cytokine immunomodulation. *Clin. Immunol.* 24: 579–590.
41. Sela, M., and E. Mozes. 2004. Therapeutic vaccines in autoimmunity. *Proc. Natl. Acad. Sci. USA* 101: 14586–14592.