Vaccination with Recombinant NY-ESO-1 Protein Elicits Immunodominant HLA-DR52b-restricted CD4+ T Cell Responses with a Conserved T Cell Receptor Repertoire

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Abstract

Purpose: ESO is a tumor-specific antigen with wide expression in human tumors of different histologic types and remarkable spontaneous immunogenicity. We have previously shown that specific T₄₁ and antibody responses can be elicited in patients with no detectable preexisting immune responses by vaccination with rESO administered with Montanide ISA-51 and CpG ODN 7909. The purpose of the present study was to characterize vaccine-induced ESO-specific CD₄⁺ T cell responses.

Experimental Design: We generated CD₄⁺ T cell clones from patient C2, who had the highest CD₄⁺ T cell response to the vaccine, and analyzed their fine specificity and HLA class II restriction to determine the recognized epitope. We then assessed the response to the identified epitope in all vaccinated patients expressing the corresponding HLA class II allele.

Results: We found that ESO-specific CD₄⁺ T cell clones from patient C2 recognize peptide ESO₁₁₉-₁₄₃ (core region 123-137) presented by HLA-DR₅₂b (HLA-DRB₃*0202), a MHC class II allele expressed by about half of Caucasians. Importantly, following vaccination, all patients expressing DR₅₂b developed significant responses to the identified epitope, accounting for, on average, half of the total CD₄⁺ T cell responses to the 119-143 immunodominant region. In addition, analysis of ESO-specific DR₅₂b-restricted CD₄⁺ T cells at the clonal level revealed significant conservation of T cell receptor usage among different individuals.

Conclusions: The identification of a DR₅₂b-restricted epitope from ESO that is immunodominant in the context of vaccine-elicited immune responses is instrumental for the immunologic monitoring of vaccination trials targeting this important tumor antigen.

To complement standard therapy, active elicitation of tumor-specific immune responses through vaccination, ideally in combination with immunomodulation, is presently viewed as a strategy that could potentially prevent disease recurrence or/

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Identification of immunodominant tumor antigen–derived CD4+ T cell epitopes restricted by frequently expressed HLA class II molecules is instrumental for the immunologic monitoring of tumor antigen–based vaccine trials, allowing for assessment of correlates between immune responses and clinical outcomes. Whereas many CD4+ T cell epitopes restricted by HLA-DR molecules encoded by the highly polymorphic DRB1 gene have been characterized, only few epitopes restricted by molecules encoded by the less polymorphic DRB3, DRB4 or DRB5 genes have been identified thus far. Here, we have characterized CD4+ T cell responses induced by vaccination with a recombinant NY-ESO-1 (rESO) protein and identified an ESO-derived, DR52b (DRB3*0202)–restricted, CD4+ T cell epitope. The identified epitope is immunodominant, as specific responses were detectable in all vaccinated patients expressing DR52b, and is recognized by CD4+ T cells exhibiting conserved T cell receptor usage in different individuals.

In a recent vaccination trial using as immunogen rESO administered with Montanide ISA-51 and CpG ODN 7909 to patients with no detectable preexisting immunity to ESO, we obtained induction of significant T11 ESO-specific CD4+ T cell responses in all patients (9). Analysis of the fine specificity of vaccine-induced CD4+ T cell responses, we detectable CD8+ and CD4+ T cell responses, we

Materials and Methods

Patients samples, cells, and tissue culture. Peripheral blood samples were collected from cancer patients enrolled in a clinical trial of vaccination with recombinant ESO protein, Montanide ISA51, and CpG 7909 (9) upon informed consent and approval by the Institutional Review Boards of Columbia University and New York University medical centers. Study patients received four s.c. injections of rESO/Montanide/CpG vaccine at 3-wk intervals. The patients enrolled had histologic diagnosis of cancer types known to express ESO. Of the 18 patients enrolled in the clinical trial, 11 were diagnosed with melanoma, 3 with breast cancer, 3 with sarcoma, and 1 with ovarian cancer. At study entry one sarcoma patient had a lung metastasis and all other patients had no evidence of disease. With the exception of one melanoma patient, none of the patients had detectable ESO-specific immune responses prior to vaccination, but they all developed specific antibody and CD4+ T cell responses following vaccination, as reported previously (9). Peripheral blood samples from healthy donors were obtained from the Etablissement Français du Sang Pays de la Loire (Nantes, France). MHC class II alleles were determined by high-resolution molecular typing. Melanoma cell lines were kindly provided by Dr. D. Rimoldi (Ludwig Institute for Cancer Research, Lausanne, Switzerland) and Prof. F. Jotereau (INSERM U892, Nantes, France). Monocyte-derived dendritic cells (moDC) were generated from enriched CD14+ cells, isolated from peripheral blood mononuclear cells (PBMC) using magnetic sorting (Miltenyi Biotech Inc.), by culture in the presence of 1,000 U/mL rhGM-CSF and 1,000 U/mL rhII-4 (R&D Systems) for 5 d.

Assessment of ESO-specific CD4+ T cell responses and generation of specific clones. For ex vivo assessment, cryopreserved total PBMC were thawed, rested overnight, and stimulated for 7 h in the absence or presence of a pool of 20 to 24 amino acid long overlapping peptides (NeoMPS Inc.) spanning the full-length ESO sequence. Brefeldin A was added 2 h after the beginning of the incubation. Cells were then stained with antibodies directed against surface markers (CD3, CD4, and CD8; BD Biosciences), fixed, permeabilized, and stained with anti-IFN-γ, IL-4, IL-10 (BD Biosciences), or -IL-17 monoclonal antibodies (mAb; eBiosciences), as previously described (9). For assessment of CD4+ T cell responses following in vitro stimulation, CD4+ cells were enriched from PBMC by magnetic cell sorting (Miltenyi Biotec Inc.) and stimulated with irradiated autologous antigen-presenting cells (APC) in the presence of the NY-ESO-1 peptide pool or the indicated NY-ESO-1 peptides (2 μmol/L each; NeoMPS Inc.), IL-2 (10 IU/mL), and IL-7 (10 ng/mL). At day 8 cultures were tested for intracellular IFN-γ secretion following stimulation, 4 h in the absence or presence of the ESO peptide pool or of individual peptides. Where indicated, CD4+ T cell cultures were preincubated for 1 h with anti-HLA-DR/IL-52 mAb (clone 7.3.19.1; Monosan) prior to peptide stimulation. ESO119–143–specific CD4+ T cells were isolated, following 4 h stimulation, using the IFN-γ secretion assay – cell detection kit (Miltenyi Biotec Inc.) and flow cytometry cell sorting and cloned by limiting dilution cultures in the presence of phytohemagglutinin, allogeneic irradiated PBMC, and rhIL-2 (100 IU/mL). Clones were subsequently expanded by periodic stimulation (every 3-4 wk) under the same conditions.

Antigen recognition assays and TCR BV analysis. CD4+ T cell clones were stimulated in the absence or presence of peptides, at the indicated concentration, and IFN-γ production was assessed in a 4-h intracellular cytokine staining assay as described above or by measurement of IFN-γ in 24-h culture supernatant by ELISA as previously described (7). Where indicated, EBV-B cell lines or PBMC from healthy donors were preincubated in the absence or presence of peptide ESO119–143, washed, and used to stimulate CD4+ T cell clones. Blocking experiments were done by preincubating CD4+ T cells with anti-HLA-DR (clone G46-6; BD Biosciences), -DP (clone B7/21; Abcam), -DQ (clone SPV13; Immunotech), or -DRw52 mAb, prior to peptide stimulation. For assessment of reactivity to naturally processed full-length ESO, tumor cell lines or moDC were either incubated for 16 h with recombinant ESO or Melan-A proteins or transfected by electroporation with ESO-encoding pCDNA3.1 vector (Amaza Inc.) and used to stimulate CD4+ T cell clones. TCR variable β chain (BV) usage was determined by flow cytometry using anti-BV mAb (Immunotech) and by molecular analysis as described previously (10) using a panel of previously validated primers (11) and nomenclature according to Arden B. et al. (12).

Results

Isolation of ESO119–143–specific vaccine-induced T11 clones. Following vaccination with rESO, all patients developed a specific CD4+ T cell response (9). For patient C2, vaccine-induced IFN-γ–producing CD4+ T cells were detected in postimmune but not in preimmune PBMC in response to stimulation with a pool of overlapping peptides covering ESO (Fig. 1A). ESO-specific IFN-γ–producing CD4+ T cells...
represented ex vivo close to 1% of total CD4+ T cells and displayed a typical Th1 profile, as they failed to produce IL-4, IL-17, or IL-10 in response to antigen stimulation (data not shown). We isolated specific CD4+ T cells based on IFN-γ secretion, followed by cloning under limiting dilution conditions as described (2). We obtained four clones reactive to the ESO peptide pool and tested them for reactivity to immunodominant peptides ESO81-100 and ESO119-143. The clones specifically recognized peptide ESO119-143, but not ESO81-100 (Fig. 1B and C). As determined by using a panel of BV-specific mAb, all clones used BV2 (Fig. 1D).

**Peptide ESO119-143 is recognized by vaccine-induced CD4+ T cells in the context of HLA-DR52b.** To determine the HLA-restriction of vaccine-induced ESO119-143-specific clones from patient C2, we first assessed inhibition of antigen recognition using blocking mAb against HLA-DR, -DP, and -DQ. For all clones, antigen recognition was inhibited in the presence of anti-DR but not anti-DP and anti-DQ mAb (Fig. 2A). As assessed by high-resolution molecular typing, patient C2 expressed DRB1*0701, DBR1*1201, DRB3*0202, and DRB4*0103 alleles. We first tested the clones for their capacity to recognize peptide ESO119-143 presented by transfected mouse fibroblasts expressing DRB1*0701 (L-DR7 cells), and detected no reactivity (data not shown). We could not directly assess restriction by DRB1*1201 as no DRB1*1201+ APC were available. To establish the frequency of the restricting allele in the population, we assessed the ability of APC from HLA-unselected healthy donors to present peptide ESO119-143 to clone C2/C4E7. This analysis revealed that APC from 8 of 15 donors were able to present the antigen (Fig. 2B). Therefore, the frequency of the restricting allele (50%) did not correspond to the frequency of DRB1*1201 in the population (about 3%), leaving DRB3 and DRB4 molecules, that are less polymorphic than DRB1, as possible candidates. In line with this, a monoclonal antibody specific for HLA-DR52 abrogated antigen recognition by clone C2/C4E7 but not by a control CD4+ T-cell clone (672/33) recognizing an unrelated peptide (SSX-237-58) in the context of DR11 (ref. 13; Fig. 2C). To define the restricting allele, we used as APC molecularly typed EBV-immortalized B cell lines EBV14 [DRB3*0202 (DR52b)], COX [DRB3*0101 (DR52a)], and EBV156 [DRB3*0301 (DR52c)]. CD4+ T cells recognized peptide ESO119-143 presented by EBV14, but not by COX or EBV156, thus establishing DRB3*0202 (DR52b) as the restricting allele (Fig. 2D). **ESO123-137 is the minimal optimal peptide recognized by DR52b-restricted CD4+ T cell clones.** To define the DR52b epitope within the ESO119-143 region, we used the MHC class II peptide prediction algorithm RankPep (http://imed.med.ucm.es/Tools/rankpep.html) to identify ESO sequences with significant predicted binding capacity to DR52b. Only two 9-mer core sequences were identified (Table 1). In particular, peptide ESO127-135 was predicted to bind DR52b with an affinity only 3-fold inferior to that of the consensus sequence.
Based on the identification of ESO127-135 as the putative core region, we designed truncated peptides by sequential removal of amino acids at either the NH2- or COOH-terminus of the original 24-mer and assessed their relative recognition efficiency by peptide titration (Fig. 3). Removal of amino acids up to position 123 at the NH2-terminus did not significantly affect recognition, whereas further truncation significantly decreased recognition. At the COOH-terminus, truncation up to position 137 did not affect recognition, whereas further truncation decreased it. These results identified the 15-mer ESO123-137 as the minimal optimal peptide recognized by DR52b-restricted CD4+ T cells.

DR52b-restricted CD4+ T cell clones recognize natural ESO antigen exogenously processed by APC. To assess the recognition of natural ESO antigen by DR52b-restricted CD4+ T cells, we tested their ability to recognize rESO processed by professional APC (DR52b+ moDC; Fig. 4A, left panel). MoDC efficiently processed rESO and presented the DR52b-restricted epitope to specific CD4+ T cells (Fig. 4A, right panel). To assess if DR52b-restricted CD4+ T cells could also directly recognize the ESO antigen endogenously expressed by tumor cells, we selected two ESO+ DR52b+ melanoma cell lines (Me252 and Me312; ref. 14). Both lines expressed significant levels of DR52 (Fig. 4B) and presented peptide ESO119-143 to specific CD4+ T cells (Fig. 4C). However, we failed to detect significant direct recognition of tumor cells by ESO-specific DR52b-restricted CD4+ T cells even after treatment with IFNγ (Fig. 4C). Similarly, A2+DR52b+ moDC, transfected with a plasmid encoding ESO, failed to be recognized by specific DR52b-restricted CD4+ T cells, although they were recognized by A2-restricted CD8+ T cells (Fig. 4D). Thus, ESO119-143-specific DR52b-restricted CD4+ T cells were able to recognize exogenously but not endogenously processed ESO antigen.

**Table 1. Ranking and score of putative ESO sequences predicted to bind DR52b**

<table>
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<td>TA AD HRQL Q</td>
<td>1.838</td>
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*Ranking and score were calculated using the binding prediction algorithm RankPep (http://imed.med.ucm.es/Tools/rank-pep.html).

1 % Optimal = (score indicated peptide/score consensus reference sequence YIKGRKPI) × 100.
**ESO119-143**-specific DR52b-restricted CD4+ T cell responses are immunodominant following vaccination with ESO protein. To evaluate the prevalence of ESO-specific DR52b-restricted CD4+ T cell responses in patients vaccinated with rESO, we isolated CD4+ T cells from postimmune samples of 15 patients and stimulated them during 10 days with the pool of ESO peptides. We then assessed the presence of specific CD4+ T cells by intracellular IFN-γ staining after stimulation with peptide ESO119-143. To determine the proportion of DR52b-restricted CD4+ T cells in the cultures, we did the assay in the absence or presence of anti-DR52 specific antibody. Six of the analyzed patients expressed DR52b and nine were negative. Significant proportions of CD4+ T cells specifically producing IFN-γ in response to ESO119-143 were detected in postvaccine samples from all patients (Fig. 5A). Their frequency in cultures from different patients ranged from 1.8% to 18.3% of CD4+ T cells and was similar, in average, for DR52b+ and DR52b- patients. However, whereas no significant inhibition of antigen recognition was observed for cultures from DR52b+ patients in the presence of the anti-DR52 mAb, the latter blocked antigen recognition by ESO119-143-specific CD4+ T cells in cultures from all DR52b+ patients, to different extents (range, 21-88%; mean 44.7% ± 23.8%; Fig. 5B). Together, our results show that DR52b-restricted ESO119-143-specific CD4+ T cell responses are immunodominant in DR52b-expressing patients vaccinated with the rESO.

**Conserved TCR usage of ESO119-143**-specific DR52b-restricted CD4+ T cell clones. T cell clones recognizing defined MHC/peptide complexes can display conserved structural features. To assess TCR usage of clones recognizing peptide ESO119-143 in the context of DR52b, we derived a panel of ESO119-143-specific clones from vaccinated patients expressing DR52b. We obtained 62 clones from 4 different patients (50 clones from patient C2, 8 from patients N13, 3 from C5, and 1 from patient N10). Of these, 33 (53%) were DR52b-restricted, as determined by using molecularly typed APC (data not shown). Because the CD4+ T cell clones initially obtained from patient C2 used BV2, we assessed BV2 expression by all other clones using specific mAb. This analysis revealed that >70% of the DR52b-restricted clones (including clones from three patients) expressed BV2. To further assess the structural features of DR52b-restricted TCR, we sequenced the TCR β chains of the BV2-expressing clones. We identified 13 distinct clonotypes (Table 2), 5 of which used the same TCR β chain joint segment (2.1) whereas the remaining 8 used 6 other BJ. In addition, the 13 distinct CDR3 regions were variable both in terms of length (10-13 amino acids) and amino acid composition. Some conservation was nevertheless noticeable, such as the presence of A at position 1 of the CDR3 of 11 of the 13 clonotypes and R at position 2 for 7 of them.

**Discussion**

Here, we have reported the identification of an ESO-derived DR52b-restricted epitope recognized by CD4+ T cells induced by vaccination with a rESO vaccine administered with the immunologic adjuvants Montanide ISA-51 and CpG ODN 7909, a formulation that predominantly elicits Th1 responses. Previous studies from us and others have identified ESO119-143 as an immunodominant region, recognized by CD4+ T cells from virtually all patients with spontaneous or vaccine-induced immunity to ESO (3, 5–7). Several overlapping epitopes contained within the ESO119-143 region and restricted by multiple HLA-DR alleles have been identified (refs. 3, 4; summarized in Cancer Immunity Peptide Database). Surprisingly, the DR52b-restricted epitope identified in this study has not been reported thus far. Our group, however, has previously reported the identification of two other DR52b-restricted epitopes from the tumor antigens SXX-4 and Melan-A (16, 17).

At variance with the β chain of the mouse I-E molecule (homolog to human HLA-DR), encoded by a single gene, the β chain of human HLA-DR is encoded by multiple genes. In addition to the DRB1 gene encoding the prevalent β chain of the DR isotype, additional genes code for other β chains, namely DRB3 (DR52), DRB4 (DR53), and DRB5 (DR51). They are less polymorphic than DRB1 and generally expressed at lower levels, but code for DR molecules that are fully functional with respect to antigen presentation (18, 19). These genes have strong linkage disequilibrium with defined DRB1 alleles. In particular, DR52 is very frequently expressed in the population, as DRB3 alleles are associated through linkage disequilibrium to some of the most common DRB1 alleles (20). Lower expression and linkage disequilibrium with DRB1 alleles may account for the fact that T cell epitopes restricted by these alternate DR molecules have been described less frequently than those restricted by DRB1-encoded molecules, or have been reported as restricted by the associated DRB1 allele.

In general, alternate DR molecules have been less well investigated as compared with those encoded by DRB1. However, expression of several DRB3-encoded molecules has been recently reported to associate with different autoimmune diseases, which has resulted in an increased interest in investigating their structure and peptide-binding specificity. Expression of DRB3*0202 (DR52b), one of the main DRB3

![Fig. 3. Determination of the minimal sequence optimally recognized by ESO119-143-specific CD4 T cell clones. Clone C2/C4E7 was stimulated, in the presence of EBV14, with serial dilutions of the indicated truncated peptides, IFN-γ was measured in culture supernatants by ELISA (upper panel), and peptide activity was calculated relative to that of ESO119-143 (lower panel).](image-url)
alleles, has been associated with Grave’s disease, multiple sclerosis, and essential hypertension caused by infection with *Chlamydia pneumoniae* (21–23).

Using truncated overlapping peptides, we defined the minimal optimal sequence recognized by ESO DR52b-restricted CD4+ T cells as the 15-mer 123-137. Within this sequence, a screening of the entire ESO sequence, using the MHC class II peptide-binding prediction algorithm RankPep (http://imed.med.ucm.es/Tools/rankpep.html), identified a sequence with high predicted binding capacity to DR52b, corresponding to peptide 127-135 (TVSGNILTI). Although the crystal structure of DR52b has not been yet resolved, some structural consideration on the potential contribution of single amino acids in the identified peptide to binding can be drawn based on previous analyses of natural peptides isolated from DR52 molecules and on the recently reported structure of the highly homologous DR52c molecule bound to a self-peptide derived from the Tu elongation factor (24, 25).

The most salient feature of the identified peptide is the amino acid N located in the central part of the sequence. Together with DR52c, and at variance with most other DR molecules, DR52b has a Q at position b74, that together with other residues in the P4 pocket, limits the amino acids binding at this position to N or D, whereas the P1 and P6 pockets are expected to be rather permissive and can accommodate many different residues.

Fig. 4. Recognition of naturally processed ESO protein by DR52b-restricted CD4+ T cells. A, surface expression of DR52 on DR52b+ moDC was assessed by staining with specific mAb and flow cytometry analysis (left panel). Clone C2/C4E7 was stimulated with moDC pulsed with ESO or Melan-A recombinant proteins at the indicated concentrations and IFN-γ was measured in culture supernatants by ELISA (right panel). B, surface expression of DR52 on DR52b+ tumor lines was assessed as in (A), following 24-h culture in the absence or presence of IFN-γ (500 IU/mL). C, tumor cell lines, cultured in the absence or presence of IFN-γ for 24 h, were preincubated in the absence or presence of peptide ESO19-143 and used to stimulated clone C2/C4E7. IFN-γ was then measured in culture supernatants by ELISA. D, A2*DR52b+ moDC were transfected with full-length ESO encoding plasmid or incubated with the indicated peptide and used to stimulate DR52b- or A2-restricted clones. IFN-γ was measured in 24-h culture supernatants by ELISA.

Fig. 5. Induction of DR52b-restricted ESO19-143-specific CD4+ T cell responses following vaccination with ESO protein. A, % of IFN-γ-producing CD4+ T cells in response to peptide ESO19-143 in postvaccine cultures from DR52b+ and DR52b- patients assessed by intracellular cytokine staining. B, % IFN-γ-producing CD4+ T cells in the same cultures as in (A) were assessed in the absence or presence of anti-DR52 mAb. % inhibition = 100 - (% IFN-γ+ CD4+ T cells in presence of mAb/% IFN-γ+ CD4+ T cells in absence of mAb) × 100. Mean % inhibition for all DR52b+ and DR52b- patients is shown.
The prevalence of DR52b-restricted CD4+ T cells in patients different and remains to be determined.

With spontaneous responses to ESO, however, might be absence, in lysosomes (30). Generation of ESO-specific CD4+ T pathway, as they are localized in melanosomes, or, in their endogenous MHC class II processing and presentation at variance with cancer/testis antigens, melanocyte differen-
tumor antigens, depending on their intracellular localization. The prevalence of DR52b-restricted CD4+ T cells in patients with spontaneous responses to ESO, however, might be different and remains to be determined.

ESO-specific DR52b-restricted CD4+ T cell clones isolated in this study efficiently recognized the natural exogenous ESO antigen after processing and presentation by APC but failed to recognize endogenously expressed ESO. We have previously obtained similar results with CD4+ T cell clones specific for another cancer/testis antigen, SSX-4 (16), whereas we have observed recognition of both exogenous and endogenously expressed antigen using Melan-A–specific CD4+ T cells (17). The ability of CD4+ T cells to recognize endogenously expressed tumor antigens may be epitope-dependent (28, 29) and can significantly vary for different endogenously expressed tumor antigens may be epitope-dependent (28, 29) and can significantly vary for different endogenously expressed tumor antigens may be epitope-dependent (28, 29) and can significantly vary for different.

By assessing the TCR of ESO119-143-specific DR52b-restricted CD4+ T cell clones from different individuals, we could show conserved TCR usage, with frequent usage of BV2 often in association with BJ 2.1. The CD3 region of the different clonotypes identified, however, was variable, both in terms of length and amino acid composition, which could indicate a certain degree of heterogeneity in the fine specificity and/or avidity of antigen recognition among different clones. We have previously reported conserved but distinct TCR usage for ESO-specific HLA-A*0201-restricted CTL that occur naturally or are induced through peptide vaccination (34), which was associated with their ability to recognize or not the naturally processed antigen. To our knowledge, however, this is the first study assessing TCR usage by ESO-specific CD4+ T cells. It will therefore be of interest to compare the TCR usage of ESO119-143-specific DR52b-restricted CD4+ T cell clones elicited by vaccination with that of CD4+ T cells naturally occurring in patients with spontaneous immunity to ESO.

We are grateful to the clinical research teams at New York University and Columbia University as well as the members of the Ludwig Institute Clinical Trial Office who have been involved in conducting the previously reported clinical study (9).

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

**Acknowledgments**

Table 2. Analysis of CDR3 β sequence and length of BV2+ ESO119-143–specific DR52b-restricted CD4+ T cell clones

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*Nomenclature used is according to Arden B. et al. (12).
References


21. Chien QY, Huang W, She JX, Baxter F, Volpe R, Maclaren NK. HLA-DRB1*08, DRB1*03/DRB3*0101, and DRB3*0202 are susceptibility genes for Graves’ disease in North American Caucasians, whereas DRB1*07 is protective. J Clin Endocrinol Metab 1999;84:3182 - 6.


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