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On T-cell Recognition of Nickel as a Hapten

LENNART EMTESTAM[1] and OLLE OLERUP[2]

[1]Department of Dermatology, Huddinge Hospital, Huddinge and [2]Center for BioTechnology, Karolinska Institute, NOVUM, Huddinge and Department of Clinical Immunology, Huddinge Hospital, Huddinge, Sweden

T-cells recognize antigens as peptides associated with self-molecules encoded by genes of the HLA region. In patients with contact allergy to nickel, T-cells that are specific for non-peptide haptens have been described. Previously, we have isolated HLA class II-restricted nickel-specific T-cell clones from patients with nickel sensitivity. In this paper, data on the fine specificity of a nickel-specific HLA-DR4-restricted clone have been reevaluated. Genomic tissue typing employing polymerase chain reaction and sequence-specific primers were used. Nickel was presented to the T-cell clone by all three subtypes of HLA-DR4 included in our panel. Two different *DRB4*0404*-positive cells presented nickel, whereas only 3 of the 7 *DRB1*0401*-positive and one of the 3 *DRB1*0408*-positive cells restimulated the T-cell clone. These findings are compatible with the notion that nickel interacts with endogenous peptides in the antigen-presenting groove of the HLA molecule, thereby changing these peptides' antigenicity rather than their ability to bind to the HLA molecule. Variations of the endogenous peptide in the antigen-presenting groove as well as differences of the HLA molecules give the DR4 specificity of the nickel-specific clone MCE2. ***Key words: antigen presentation; HLA-DR; major histocompatibility complex; nickel contact dermatitis.***

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L. Emtestam, Department of Dermatology and Venereology, Huddinge Hospital, S-141 86 Huddinge, Sweden.

The HLA genes encode polymorphic cell surface structures, which have two major functions. Firstly, they are markers for self during the positive and negative selection of maturing T-cells in the thymus and secondly, the HLA molecules serve as restriction elements for presentation of antigenic peptides, i.e. they participate in the cellular interactions responsible for a T-cell dependent immune response.

Specific T-cell receptors do not recognize native proteins but respond to peptide fragments of antigens in conjunction with HLA molecules (1, 2). A trimolecular complex is formed, consisting of peptide, HLA molecule, and T-cell receptor. In the case of non-peptide haptens this complex is still undefined but can be studied indirectly with e.g. nickel-specific T-cell clones (3–5). We have studied such proliferative clones, restricted by HLA class II molecules, using a panel of carefully HLA-typed donors of antigen-presenting cells (4, 5). However, the fine specificity of these nickel-specific clones could not be completely assessed in the previous studies. In recent work, results have indicated that interactions occur between the nickel ions and the endogenous peptides located in the antigen-presenting groove of the HLA molecule, thereby altering the immunogenicity of those peptides (6). In this study, we have reevaluated our previous results with a nickel-specific clone MCE2 (4). Since this clone was found to be restricted by DR4, we have in the present report tried to assess the fine specificity of the HLA class II restriction molecules by subtyping with polymerase chain reaction (PCR) and sequence-specific primers (SSP).

MATERIAL AND METHODS

T-cell clone

The derivation and characterization of the CD4-positive, HLA-DR4-restricted nickel-specific T-cell clone MCE2 have been described elsewhere (4).

Cell donors

Healthy individuals of a well-defined HLA-typed population served as donors of peripheral blood mononuclear cells. To reveal the fine specificity of the DR4 restriction of the clone MCE2, only donors that were DR4-positive were included.

DNA-extraction

Whole EDTA-treated peripheral blood (5 ml) was deep frozen (−70°C) until use. High molecular weight DNA was extracted from proteinase-K-treated blood leukocytes by phenol/chloroform extractions or by salting-out in mini-scale (7, 8).

Southern blot analysis

Restriction enzyme digestion (*TaqI*), agarose gel electrophoresis, capillary blotting to nylon membranes, hybridization, stringency washes and autoradiography were performed by standard techniques with minor modifications (9).

TaqI HLA-DR and -DQ RFLP analysis

Genomic DR and DQ typing was performed by hybridizing *TaqI*-cleaved DNA with *DRB*, *DQA* and *DQB* probes. Allelic restriction fragment patterns were designated by the associated serologically defined DR and DQ specificities according to a previous report (10).

*Primers for subtyping of the DRB1*04 alleles*

A set of sequence-specific primers and appropriate control primers have been designed in our laboratory to discriminate the presently known *DRB1*04* variants, *DRB1*0401–0412*. For details on names, nucleotide sequences, lengths and specificities of the PCR primers, see ref 11. The amplification primers were synthesized on a 380B DNA synthesizer (Applied Biosystems) and purified by high-pressure liquid chromatography (Hitachi).

PCR reaction mixtures

Each PCR reaction mixture contained two allele- or group-specific primers, as well as an internal positive control primer pair. The PCR reaction mixtures (10 µl) consisted of 75 ng genomic DNA, PCR buffer (50 mM KCL/1.5 mM $MgCl_2$/10 mM Tris-Cl, pH 8.3/001% (w/v) gelatin), 200 µM each of dATP, dCTP, dGTP and dTTP, 0.25 µM of the allele- and group-specific primers, 0.08 µM of the control primers and 0.25 unit of AmpliTaq (Perkin Elmer Cetus Corporation).

PCR cycling parameters

Thirty amplification cycles were carried out in a GeneAmp PCR System 9600 thermal cycler (Perkin Elmer Cetus Instruments). Each cycle consisted of denaturation at 94°C for 20 s, annealing at 65°C for 50 s and extension at 72°C for 20 s.

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Agarose gel electrophoresis

Absence or presence of PCR products was visualized by agarose gel electrophoresis. After addition of 2 μl loading buffer (30% (v/v) glycerol stained with bromophenol blue and xylene cyanol), the PCR reaction mixtures were loaded in 3-mm-wide slots in 2% (w/v) ME agarose gels (SeaKem, FMC BioProducts) prestained with ethidium bromide (0.5 μg/ml gel). Gels (20 × 20 cm) were run for 15 min at 10 V/cm in 0.5 × TBE buffer (89 mM Tris base/89 mM boric acid/2 mM EDTA, pH 8.0) without buffer recirculation. Gels were examined under UV illumination and documented by photography.

Restimulation and assay of DNA synthesis

Cultures were set up in round bottom microtitre plates (Nunc, Denmark) in triplicate, each well containing 1–2.5 × 10^4 cloned T-cells and 10^4–10^5 irradiated (35 Gy) autologous or allogeneic peripheral mononuclear cells with or without nickel sulfate in the medium. The plates were incubated at 37°C in humified air containing 5% CO_2 for 3 days. To each well 1 μCi of (methyl-^{3}H)-thymidin (Amersham International Plc, Amersham, England, spec act 5 Ci/mmol), contained in 0.05 ml, was added 18 h before harvesting. Harvesting was carried out on glass fibre filters employing a Titertech cell harvester (Flow Lab., Irvine, Scotland). Filters were dried and assayed in an LKB liquid scintillation counter (Wallac 1216 Rackbeta II, Finland). Results were expressed as mean counts per minutes. A response was considered positive when it was more than 40% of the response of the autologous cell combination.

RESULTS

The specificity of the HLA class II restriction of the nickel-specific T-cell clone MCE2 (4) was reanalyzed. The results are based on the capacity of the clone to respond to nickel presented by allogeneic antigen-presenting cells from a panel of genomically HLA-typed individuals. Since MCE2 is known to be restricted by DR4, in this report only DR4-positive cell donors were reevaluated, and subtyping of HLA-DR4 was performed in these cell donors as well as the donor of the clone. Only the result of the HLA-DR4 genotyping is reported here, i.e. *HLA-DRB1*0401* to **0412*.

The results of the high-resolution typing of the DRB1*04 group of alleles as well as the proliferative responses are given in Table I. The clone MCE2 was, as expected, restimulated in the autologous combination and the T-cell clone donor was typed as *DRB1*0404*. Looking at the allogeneic combinations, both donors that were *DRB1*0404*-positive, 3 out of 7 of the *DRB1*0401*-positive and one out of 3 of the *DRB1*0408*-positive cells restimulated the T-cell clone. The restimulation of *DRB1*0401*- and *DRB1*0408*-positive donors was not influenced by the closely linked DQ genes (Table I). The magnitude of the proliferative response was approximately the same for the 3 *DRB1*04* alleles. There were no individuals found with the, in Sweden relatively rare, genotypes *DRB1*0402-0403*, *DRB1*0405-0407* and *DRB1*0409-0412*. One DR4-positive cell gave a proliferative response in the absence of nickel.

The results indicate that with this clone the most consistent finding was that both *DRB1*0404*-positive individuals responded, but for *DRB1*0401*– and *DRB1*0408*– positive cells the restimulations varied. In Fig. 1 the amplification patterns of the T-cell clone donor as well as 3 subjects of the panel obtained by *DRB1*04* high-resolution typing using SSP are depicted.

DISCUSSION

Nickel-specific T-cell clones are appropriate for studies on the mechanisms involved in the regulation of hapten-induced immune responses. In 1985, Sinigaglia et al. isolated nickel-specific clones, and in studies using four such clones and antigen-presenting cells from 6 donors, these were found to be DR-restricted (3). Kapsenberg et al. have also produced nickel-specific clones, but from dermal lymphocytic infiltrates

Table I. *HLA-DR restriction specificity of the nickel-specific T-cell clone MCE2*

	Class II serology		RFLP-typing[a]		*DRB1*04*-subtyping[b]	Response to nickel	
	DR	DQ	DR	DQ			(%)
Autologous	3, 4	2, 3	4, 17	2, 8	*0404*	Pos[c]	(100)
Allogeneic							
1.	2, 4	1, 3	4, 15	6, 7	*0401*	Pos	(82)
2.	4, 13	1, 3	4, 13	6, 8	*0401*	Pos	(65)
3.	4, 13	1, 3	4, 13	6, 8	*0401*	Pos	(78)
4.	4, 8	3, x	4, 8	4, 7	*0401*	Neg	(5)
5.	2, 4	1, 3	4, 15	6, 8	*0401*	Neg	(8)
6.	4, 8	3, x	4, 8	4, 8	*0401*	Neg	(15)
7.	4, 8	3, x	4, 8	4, 8	*0401*	Neg	(23)
8.	4, 13	1, 3	4, 13	6, 8	*0401*	AR[d]	(75)
9.	1, 4	1, 3	1, 4	5, 8	*0404*	Pos	(68)
10.	3, 4	2, 3	4, 17	2, 8	*0404*	Pos	(88)
11.	4, 13	1, 3	4, 13	6, 7	*0408*	Pos	(90)
12.	1, 4	1, 3	1, 4	5, 7	*0408*	Neg	(18)
13.	4, 13	1, 3	4, 13	6, 8	*0408*	Neg	(20)

[a] The DR and DQ specificities associated with specific *Taq*I RFLP patterns are given in the table (ref 10). (RFLP typing defines more polymorphism than serology, e.g. DR3 can be separated into DR17 and DR18.)

[b] The serologically defined DR4 specificity can be subdivided into 12 nucleotide sequence-defined alleles, named *DRB1*0401* to *0412* and not related to the serologically defined DR specificities, DR1, DR2 etc.

[c] Positive response was defined as more than 40% of the response in the autologous combination.

[d] AR: alloreactivity, i.e. high proliferative response in the absence of nickel.

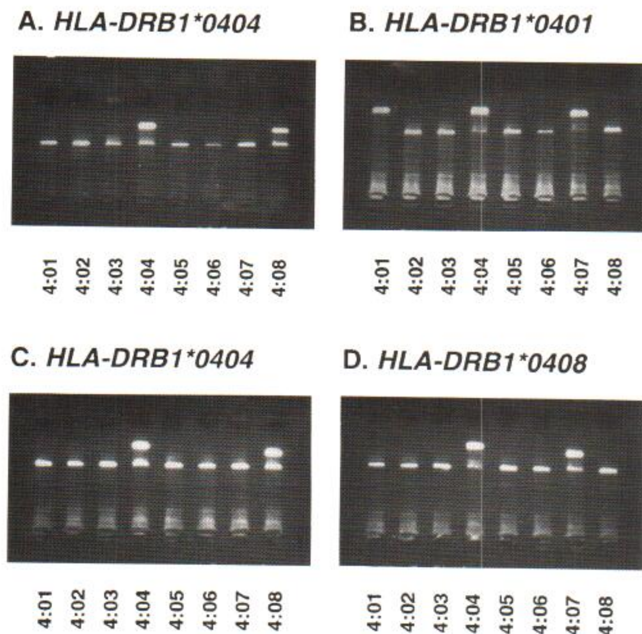

Fig. 1. Polymerase chain reaction products obtained by high-resolution typing for the DRB1*04 group of alleles, using sequence-specific primers, were size-separated on a 2% ethidium bromide-stained agarose gel. Below each lane the name of the corresponding primer mix is given (ref. 11). (Primer mixes are arbitrarily named and the numbering of them does not refer to the different *DRB1*04* alleles.) The lower band of equal length on the four gel photographs originates from an internal positive PCR control primer pair. The *DRB1*04*-specific PCR fragments are all shorter than the control band. A: patient – donor of cell line and autologous antigen-presenting cells *(DRB1*0404,* specific PCR fragments in lanes 4 and 8). B–D: donors of allogeneic antigen-presenting cells (*B: DRB1*0401*, specific PCR fragments in lanes 1, 4 and 7; C: *DRB1*0404*, specific PCR fragments in lanes 4 and 8; *D: DRB1*0408*, specific PCR fragments in lanes 4 and 7).

in nickel-positive patch tests, and these clones were discovered to be DR-restricted, as shown in blocking experiments with anti-DR monoclonal antibodies (12). In experiments using eleven different clones and cells from 23 different carefully HLA-typed cell donors as well as blocking with monoclonal antibodies directed against DR, DQ and DP molecules, we demonstrated that all clones except one were DR-restricted (4). No clone was DQ-restricted, and the remaining clone was restricted by DP (Emtestam et al., in prep.). Interesting results were obtained regarding the DR-restricted clone MCE2, which exclusively showed a proliferative response when stimulated by DR4-positive cells. However, 6 of the 14 DR4-positive cells in that study did not present nickel to the clone, implying a functional subdivision of the serological specificity DR4. This split did not correspond with the results of cellular Dw-typing. In view of the recent interesting discovery of Romagnoli et al. (6) on the interactions of nickel and endogenous peptides bound to the antigen-presenting groove of the HLA molecules, we have decided to reevaluate our previous restriction analysis of the MCE2 clone. In the present study, the genomic typing was extended by the use of PCR and SSP for all currently known *DRB1*04* alleles.

There are structural resemblances of all DR molecules, since they share the same alpha-chain. Also, DR1, DR4 and DR7 are highly homologous in most of their beta-chains (13). However, in this material such universal DR motifs did not seem to influence the reactivity, since the response was not influenced by the DR allele on the other haplotype and strictly DR4-positive cells restimulated the clone. A total of 14 DR4-positive cells were tested with the clone and 8 restimulated, but none of the 11 DR4-negative cells used were able to restimulate the clone (4). The specificity testing of MCE2 shows that both cells typed as *DRB1*0404* responded (Table I). This was also the DR4 genotype of the patient who donated cells for the isolation of the clone. Thus, the restriction element of MCE2 is present on cells carrying *DRB1*0404*. The results were not as clear-cut in the remaining two variants of DR4 genotypes that were found among the donor cells in this study. For the *DRB1*0401* specificity, 3 out of 7 cells restimulated and for *DRB1*0408*, one out of 3 restimulated the clone. The *DRB1*0404* and *0408* alleles differ by only one single amino acid in the membrane-distal domain of the molecule; the hydrophobic valine at position 86 of the DRbeta-chain in *DRB1**0404 is changed to the neutral glycine in *DRB1*0408*. Likewise, the *DRB1*0404* and *0401* alleles differ merely by the amino acids at positions 86 and 72, *0404* encodes valine and arginine, and *0401* encodes glycine and lysine at these positions, respectively. Although others have reported polymorphism at position 86 to be important for T-cell recognition (14, 15), this was not the case in the experiments with the clone MCE2, where the minor amino acid differences between the actual DR4 subtypes have little biological relevance. It is more important that the *DRB1*0401*, *DRB1*0404* and *DRB*0408* have shared motifs, and these shared motifs are the basis for the fact that all three DR4 types can restimulate the MCE2 clone in the presence of nickel ions. Furthermore, the clone MCE2 was also restimulated by one allogeneic cell (cell donor no. 8 in Table I) in the absence of nickel, implying that the MHC peptide complex cross-reacts with a MHC nickel-modified peptide. This finding has been described previously and is frequently seen in experimental studies on the fine specificity of hapten-specific T-cell clones (16, 17). The overall results of the specificity analysis indicate that the MCE2 clone is restricted by DR4 in combination. It is tempting to speculate that nickel modifies one set of DR4-bound peptides, or perhaps more likely, one single DR4-associated peptide (18, 19).

Recent studies on the interaction between nickel- and MHC-bound endogenous peptides have added crucial information regarding nonpeptide hapten immune responses. In vivo, the peptides are bound to the antigen-presenting groove of the HLA molecule (20–22). By the use of different model peptides, Romagnoli et al. demonstrated that nickel binds to histidyl groups of the endogenous peptide. They also proposed that the nickel ion may bind to tolerated MHC-self peptide complexes to make them immunogenic (6). Our own studies on the heterogeneic restriction response of the nickel-specific clones certainly support this concept. Each HLA molecule can bind approximately 1,000 different peptides, some of which have free histidyl groups directed towards the T-cell receptor. Our clone, MCE2, is restimulated only by those DR4 types that bind the nickel-peptide complexes. On the other hand, of clones made from the same patient as MCE2, one was restricted by DR3 and one neither by DR3 nor DR4. The fourth clone from the same patient was also restricted by DR4, but the restriction element was different from the one in MCE2 (4). There is a variation in the availability of

chemically reactive groups of the peptides, and this variation might be the mechanism responsible for the ease by which an individual becomes sensitized to nickel, as well as the regulation of the nickel-specific T-cell response. Furthermore, the same phenomenon also gives a possible explanation why no HLA association is detectable in nickel-allergic populations (23), since any given HLA molecule can bind and present an array of nickel-modified endogenous peptides.

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