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In previous studies, the shared cancer-testis Ag, NY-ESO-1, was demonstrated to be recognized by both Abs and CD8\(^+\) T cells. Gene expression of NY-ESO-1 was detected in many tumor types, including melanoma, breast, and lung cancers, but was not found in normal tissues, with the exception of testis. In this study, we describe the identification of MHC class II-restricted T cell epitopes from NY-ESO-1. Candidate CD4\(^+\) T cell peptides were first identified using HLA-DR4 transgenic mice immunized with the NY-ESO-1 protein. NY-ESO-1-specific CD4\(^+\) T cells were then generated from PBMC of a patient with melanoma stimulated with the candidate peptides in vitro. These CD4\(^+\) T cells recognized NY-ESO-1 peptides or protein pulsed on HLA-DR4\(^+\) EBV B cells, and also recognized tumor cells expressing HLA-DR4 and NY-ESO-1. A 10-mer peptide (VLLKEFTVSG) was recognized by CD4\(^+\) T cells. These studies provide new opportunities for developing more effective vaccine strategies by using tumor-specific CD4\(^+\) T cells. This approach may be applicable to the identification of CD4\(^+\) T cell epitopes from many known tumor Ags recognized by CD8\(^+\) T cells. The Journal of Immunology, 2000, 165: 1153–1159.

T cells play an important role in controlling tumor growth and mediating tumor regression. To understand the molecular basis of T cell-mediated antitumor immunity, a number of tumor Ags recognized by CD8\(^+\) T cells have been identified in melanoma as well as in other types of cancers (1, 2). These studies have led to several clinical trials using peptides derived from the molecularly defined tumor Ags (3–6). Although the clinical trial using a modified peptide derived from gp100 provided some evidence of therapeutic efficacy for the treatment of patients with metastatic melanoma (3), these studies mainly focused on the use of CD8\(^+\) T cells. Increasing evidence from both human and animal studies has indicated that optimal cancer vaccines require the participation of both CD4\(^+\) and CD8\(^+\) T cells (7, 8). Moreover, tumor-specific CD4\(^+\) T cells are required for generating protective immunity against MHC class II-negative tumor cells (9, 10). Identification of such Ags is thus important for the development of cancer vaccines as well as for our understanding of the mechanism by which CD4\(^+\) T cells regulate host immune responses.

To date, only a limited number of MHC class II-restricted tumor Ags have been identified. Several known MHC class I-restricted tumor Ags, such as tyrosinase, gp100, and MAGE-3, were demonstrated to contain MHC class II-restricted epitopes recognized by CD4\(^+\) T cells (11–15). Recently, a genetic approach was developed to identify unknown MHC class II-restricted tumor Ags by using tumor-specific CD4\(^+\) T cells (16). This has led to the identification of several mutated tumor Ags, including CDC27, triosephosphate isomerase, and LDLR-FUT (16, 17). Among them, triosephosphate isomerase is a mutated Ag that was independently identified by a biochemical approach (18).

The NY-ESO-1 gene was previously identified by Ab screening (19), and was recently identified as an MHC class I-restricted tumor Ag as well (20, 21). High titers of Abs against NY-ESO-1 were also detected from patients with cancer (22). The NY-ESO-1 cDNA encoded two gene products from two overlapping open reading frames (20). Because of its strict tumor-specific expression pattern, with the exception of expression in normal tissues, as well as its high frequency of expression in many tumors, including melanoma, breast, prostate, lung, and other cancers (19, 20, 23), NY-ESO-1 is potentially an important immune target for the development of immunotherapies for a variety of cancer types (24).

Because both CTL and Ab immune responses against NY-ESO-1 were demonstrated in patients with cancer, identification of MHC class II-restricted T cell epitopes in the NY-ESO-1 protein could be important for the development of effective cancer vaccines. In this study, we report the identification of an HLA-DR4-restricted T cell epitope from the NY-ESO-1 Ag by using HLA-DR4 transgenic mice immunized with the purified NY-ESO-1 protein and in vitro stimulation of human PBMC with synthetic candidate peptides. We show that CD4\(^+\) T cells generated from human PBMC are NY-ESO-1 specific, and recognize NY-ESO-1 peptides pulsed on HLA-DR4\(^+\) EBV B cells as well as naturally processed peptides on melanoma cells.

Materials and Methods

Purification and analysis of recombinant NY-ESO-1 protein

To construct a bacterial expression vector encoding the full-length NY-ESO-1 gene, we generated a PCR fragment by using a pair of primers, ESO-5p (5'-GCTCGGGACATATGCAGGCCGAAGGCCGGGG) containing an NdeI site and ESO-3p (5'-AAGGGGCTCGAGGCTGGGCTGGGCTGGGGCTGGGGCAGCGCCGCTCTT) containing an XhoI site. After digestion with restriction enzymes and gel purification of the PCR product, a DNA fragment encoding NY-ESO-1 was fused to DNA encoding a polyhistidine peptide in frame in pET-28a+ (Novagen, Madison, WI). A similar strategy was also used to construct an expression vector for a truncated NY-ESO-1, ESOS1–74, which contained only the first 74 aa residues. Escherichia coli strain BL21(DE3) bearing the correct plasmid construct was grown at 37°C to log
phase, then induced for protein production by adding isopropyl β-D-thio-
galactoside to a final concentration of 0.5 mM and shaking for 3 h. Frac-
tions of bacterial extract were obtained; and NY-ESO-1 was purified by
Ni²⁺ affinity chromatography. SDS-PAGE analysis of the purified protein
was performed as previously reported (25). The N-terminal sequence of the
purified protein was determined by automatic Edman degradation.

**Serum and PBMC**

Sera from patients with metastatic melanoma were stored at −80°C. Sera
of normal donors were obtained from the Blood Bank at the Clinical Center
of National Institutes of Health. The MHC class II genotype of patient TE
with metastatic melanoma was HLA-DRβ1*10401, β1*1301. The patient
was treated with the gp100:209–217(210 M) peptide plus high dose of
IL-2, and experienced an objective tumor regression.

**Detection of Abs against NY-ESO-1 protein**

About 50 ng of purified NY-ESO-1 protein diluted in 50 µl PBST (PBS
with 0.1% Tween-20) was adsorbed to each well of a 96-well MaxiSorp
plate (Nunc, Roskilde, Denmark) overnight at room temperature. Control
plates were coated with 150 ng BSA/well. Plates were blocked with 5% dry
milk in PBST for at least 2 h, washed, and were loaded with 100 µl of
diluted serum samples. All serum samples were diluted at 1/25, 1/250, and
1/2500 with 3% dry milk in PBST. Each sample at the three different
dilutions was loaded onto NY-ESO-1-coated plates as well as BSA-coated
plates. After 1-h incubation at room temperature, plates were washed and
loaded with secondary Ab (goat anti-human IgG conjugated with HRP;
Sigma, St. Louis, MO) diluted with 1% dry milk in PBST. Plates were
developed after a 0.5-h incubation, and absorbance at 450 nm was read by
using an ELISA reader (Dynatech, Chantilly, VA). A positive reaction was
defined as an OD value against NY-ESO-1 that exceeded the mean OD
value plus three times SDs of normal donors at serum dilutions of both 1/25
and 1/250. Western blot was performed as described (25) to confirm the
specificity of the Ab in a few representative sera samples.

**Cell lines and Abs**

Melanoma lines F049 and F050 were early cultures of fine needle aspirate
samples, provided by Adam Riker at the Surgery Branch of National Can-
cer Institute. All other melanoma lines and EBV B lines were generated
and maintained in RPMI 1640 (Life Technologies, Rockville, MD) sup-
pplemented with 10% FCS (Biofluids, Gaithersburg, MD). 293IMDR1 and
293IMDR4 were genetically engineered to express human invariant chain,
DMA, DMB, and DR molecules, and were cultured in RPMI 1640 sup-
pplemented with 10% FCS (16). Culture medium for murine lymphocytes
was RPMI 1640 with 0.05 mM 2-ME, 5 µg/ml IL-2, plus 10% FCS
provided by HyClone (Logan, UT). Medium used for human T cell culture
was RPMI 1640 with 0.05 mM 2-ME, 50 µg/ml IL-2, plus 10% human AB
serum, provided by Sigma. Ab-blocking experiments were performed as
previously described (15). Hybridoma HB55 and HB95 were obtained
from American Type Culture Collection (Manassas, VA). Control Ab was
purchased from Pharmingen (San Diego, CA).

**Transgenic animals and immunization procedures**

HLA-DR4 transgenic (DR4-Tg) mice were murine class II deficient, and
expressed HLA-DRα1-IE-α and HLA-DR β1*0401-IE-β chimeric mole-
cules (26). Founder mice were obtained through Paul Lehmann at Case
Western Reserve University (Cleveland, OH). Mice were inbred and main-
tained at Biocon (Rockville, MD). Female mice aged between 6 and 10 wk
were immunized with the full-length recombinant NY-ESO-1 protein.
About 50 µg of purified protein were emulsified in CFA, divided evenly,
and given to each mouse i.p. into each footpad and the base of
tail. Eleven days after the injection, mice were sacrificed, and the bi-
ilateral hind limb popliteal and the inguinal lymph nodes were harvested.
Single cell suspensions were obtained from the lymph nodes of two im-
munized animals, and followed by in vitro stimulation.

**Peptide synthesis**

Synthetic peptides used in this study were made using a solid-phase
method on a peptide synthesizer (Gilion, Worthington, OH) at the Surgery
Branch of National Cancer Institute. The purity of each peptide was eval-
uated by mass spectrometry (Bio-synthesis, Lewisville, TX).

**In vitro sensitization procedure and cytokine release assays**

Peptides at a final concentration of 10 µM were mixed with 2.5 × 10⁵ mouse lymphocytes for 1 wk before cytokine release assays were con-
ducted. For in vitro sensitization of human PBMC, 2.5 × 10⁵ cells were
pulsed with peptides at 10 µM concentration and incubated in each well of
a flat-bottom 96-well plate. After two in vitro stimulations, cells were
tested against various targets and supernatants were harvested for cytokine
release assays. Rapid expansion and cloning of human T cells were per-
scribed as described (20).

Peptide at a final concentration of 10 µM or protein at a final concen-
tration of 5 µg/ml was pulsed onto target cells. After 4-h incubation, cells
were washed in serum-free RPMI medium, and −3 × 10⁵ target cells were
incubated with the same number of TE4-1 cells overnight, and cytokine
release was measured using GM-CSF ELISA kits (R&D Systems, Minne-
apolis, MN) for human IFN-γ kits (Endogen, Woburn, MA) for mouse.
Other cytokines, such as human IFN-γ, IL-10, TNF-α, and IL-4, were
measured using ELISA kits from Endogen or R&D Systems, according to
the manufacturer’s instructions.

**Results**

**Recombinant NY-ESO-1 protein and detection of NY-ESO-1-
reactive Ab**

NY-ESO-1-reactive Abs and CTL have been reported in patients
with cancer (19, 22). It thus appeared that NY-ESO-1-specific CD4⁺ T cells might play a role in orchestrating the development of
Abs as well as CTLs against the NY-ESO-1 Ag. To identify
MHC class II-restricted CD4⁺ T cell epitopes, we began by puri-
ifying NY-ESO-1 protein from a bacterial expression system as the
starting material. To facilitate NY-ESO-1 expression and protein
purification, a CDNA fragment encoding NY-ESO-1 was fused to
a polyhistidine tag in frame located at the N terminus in the pET28
expression vector, and a high level production of recombinant pro-
tein was obtained. Several milligrams of the NY-ESO-1 protein were
puriﬁed by using a Ni²⁺-charged afﬁnity chromatography column.
The puriﬁed protein showed an apparent molecular mass of ~26 kDa on an SDS polyacrylamide gel (Fig. 1A). To conﬁrm the iden-
tity of the puriﬁed protein, N-terminal microsequencing of the protein was performed by automatic Edman degradation. All 25 aa residues obtained by Edman degradation matched the predicted
amino acid sequences (data not shown). A short version of NY-
ESO-1 containing the first 74 aa residues, ESO1–74, was also puri-
ﬁed by the same approach (Fig. 1A).

To determine whether melanoma patients developed Abs
against the NY-ESO-1 protein, sera from 88 metastatic melanoma
patients enrolled in cancer vaccine treatment protocols in the Sur-
gery Branch, National Cancer Institute, were screened. Sera from
eight normal donors were used as controls for screening. Eleven of
eighty-eight patients (13%) were found to have high titers of Abs
against NY-ESO-1 (Fig. 1C). These data were consistent with re-
sults obtained by other groups (22). To exclude the possibility that
patients’ sera reacted with a minor contaminant present in the pu-
rified NY-ESO-1 protein, Western blot was performed using rep-
resentative sera samples. Fig. 1B showed that the NY-ESO-1-reactive
sera from a patient reacted only with cell lysates from NY-
ESO-1-expressing bacteria and the purified NY-ESO-1 protein, but
not with extracts from bacteria containing the control vector.
A nonreactive serum sample was also tested (Fig. 1B, lanes 4, 5, and 6).

**Identification of putative MHC class II-restricted epitopes from
HLA-DR4 transgenic mice**

To identify CD4⁺ T cell epitopes, DR4 transgenic mice were im-
munized in the tail base and rear footpads with ~50 µg of full-
length NY-ESO-1 protein in CFA. Eleven days after the injection,
single cell suspensions obtained from bilateral hind limb popliteal
and inguinal lymph nodes of two immunized mice were prepared
and used for in vitro sensitization with synthetic peptides derived
from the NY-ESO-1 protein based on the predicted peptide-bind-
ning properties of the HLA-DR4 molecules (27).
Eight high-binding peptides containing amino acid sequence segments predicted to bind to HLA-DR4 were used for the in vitro sensitization experiments. Six days after the initial in vitro sensitization, murine lymphocytes were tested for cytokine release against human HLA-DR4-positive 1359EBV B cells alone and 1359EBV B pulsed with the corresponding peptide used for stimulation. Three peptides were recognized by murine T cells based on cytokine secretion from T cells, while other five peptides showed no recognition (Fig. 2). The ESO p116–135 showed the strongest activity among the positive peptides, suggesting that this peptide might contain an epitope presented by the HLA-DR4 molecule for T cell recognition. This peptide was thus chosen for further analysis.

Generation of human CD4+ T cells specific for NY-ESO-1

PBMCs from patient TE, who had high titered Abs against NY-ESO-1 (Fig. 1C), were used for in vitro stimulation with the ESO p116–135 peptide. After 1 wk of in vitro stimulation, PBMC from patient TE showed marked expansion. IL-2 was added in the second week of stimulation. The cell line thus established was named TE4-1, which continued growth for more than 2 wk in the presence of 20 CU/ml IL-2. The TE4-1 T cells were 90% CD4+ T cells based on FACS analysis. TE4-1 contained Th1-type CD4+ T cells as they secreted GM-CSF, IFN-γ, and TNF-α, but not IL-10 or IL-4 (data not shown). After depletion of a few percent of CD8+ T cells, the purified population of CD4+ T cells still retained its reactivity. Some T cell clones derived from TE4-1 cell line were also shown to recognize the ESO p116–135 peptide (data not shown).

TE4-1 recognized EBV B cells pulsed with the full-length NY-ESO-1 protein as well as the ESO p116–135 peptide in the context of HLA-DR4, but not with the truncated NY-ESO-1 protein containing the first 74 aa (Fig. 3A). The TE4-1 cell line was also reactive specifically with DR4-positive dendritic cells infected with adenovirus encoding NY-ESO-1, but not adenovirus encoding the green fluorescence protein (data not shown).
To test whether T cell recognition by TE4-1 was restricted by HLA-DR4, two overlapping peptides (ESO p116–135 and ESO p111–130) and a control peptide (ESO p91–110) were pulsed onto 293IMDR1 and 293IMDR4 cells in serum-free medium. Cells were washed and subsequently incubated with TE4-1 cells overnight. As shown in Fig. 3B, both peptide 116–135 and peptide 111–130 were recognized by TE4-1 in the context of HLA-DR4. Interestingly, peptide 116–135 was also capable of stimulating cytokine secretion from T cells when pulsed onto 293IMDR1 cells. No activity was detected with 293IMDR4 pulsed with the control ESO p91–110 peptide (Fig. 3B). The recognition of ESO-p116–135-pulsed 293IMDR4 was completely inhibited by an anti-HLA-DR Ab (HB55), but not by the control and anti-HLA class I Abs (HB95) (Fig. 3C). A gp100-specific CD8+ T cell line (CTL-C3G1) and an HLA-DR1-restricted CD4+ T cell line (T3–80) were used as specificity controls for the Ab blocking.

**Recognition of tumor cells by TE4-1**

Although peptide-specific CD4+ and CD8+ T cell activities can often be generated against a putative tumor Ag, in many cases tumor reactivity could not be demonstrated due to either the low affinity of the T cells or the failure of presentation of naturally processed peptides on the tumor cell surface (2). To test whether TE4-1 could recognize NY-ESO-1 epitopes naturally processed and presented by tumor cells, several melanoma lines were used as targets. The expression of NY-ESO-1 in each line was determined by RT-PCR, while the expression of NY-ESO-1 epitopes naturally processed and presented by tumor cells, T cells or the failure of presentation of naturally processed peptides on the tumor cell surface.

**Characterization of the NY-ESO-1 epitope recognized by TE4-1**

Because the two reactive peptides shared 15 aa (LPVPGV-LLKEFTVSG), the minimal length of peptide was determined by testing a series of N- and C-terminal truncated peptides. Peptides were pulsed onto DR4+ 1088 EBV B cells and tested for their ability to stimulate TE4-1 cells. The valine residue at position 128 was found to be critical for T cell recognition (Fig. 5A). The peptides with the N-terminal deletions up to leucine residue at position 123 did not affect T cell recognition, but the peptide with further deletions partially lost its ability to stimulate T cells. The leucine residue at position 123 may be a P1 anchor residue because the P1, P4, P6, and P7 residues contributed to the peptide binding to MHC class II molecules. Further deletions are required to determine the critical residues for binding to MHC class II molecules.

Although CD4+ TE4-1 cells reacted with positive peptides at a relatively high peptide concentration, we found that the ESO p119–130 peptide exhibited a higher activity and required a lower peptide concentration to achieve a similar level of T cell recognition than other two short peptides, ESO p120–130 and ESO p121–130, in a titration experiment (data not shown). Thus, we used the ESO p119–130 peptide to determine the binding affinity of the peptide recognized by TE4-1. Peptides were pulsed onto 1088EBV B cells (HLA-DR4+) as targets at different peptide concentrations. As shown in Fig. 5B, no or little T cell activity was observed at 33 nM or lower concentrations of the ESO p119–130 peptide; high activities were detected at 0.33 μM peptide concentration, and the T cell activity did not reach a plateau at a 33 μM peptide concentration. The control peptide was not recognized by TE4-1 even at a 33 μM peptide concentration.

**Discussion**

NY-ESO-1 is an important immune target because it gives rise to both humoral and cellular immune responses (19–21). Although its expression pattern is similar to Ags in the MAGE gene family, NY-ESO-1 is more frequently expressed in breast, prostate, and lung cancers than any member of the MAGE family (19, 20, 23). More interestingly, high titered NY-ESO-1-reactive Abs were frequently detected in patients with cancer (Fig. 1, B and C), while a very low percentage of patients developed high titers of Abs against the MAGE Ags or differentiation Ags such as tyrosinase, gp100, TRP-1, and TRP-2 (data not shown) (22). It is possible that Abs reacting with glycosylated proteins, such as gp100, TRP-1, and TRP-2, may not react with bacteria-derived unglycosylated proteins. However, these studies strongly suggest that NY-ESO-1-reactive CD4+ T cells may be involved in Ab production and CTL proliferation. In this study, we identified the HLA-DR4-restricted T cell epitope derived from NY-ESO-1 by the use of HLA-DR4 transgenic mice and in vitro stimulation of human PBMC with candidate peptides. To our knowledge, this is the first demonstration that T cell epitopes from NY-ESO-1 were shown to be presented by HLA-DR4 (DRB1*0401) molecule to CD4+ T cells. Because NY-ESO-1-specific Abs and CTL were detected in patients with different HLA genotypes, other CD4+ T cell epitopes presented by HLA class II molecules other than HLA-DR4 probably exist. Indeed, after this work was submitted, a study describing the identification of NY-ESO-1 epitopes presented by DRB4*0101–0103 was reported (28).
Recently, two groups reported the identification of MHC class II-restricted T cell epitopes from the known MHC class I-restricted tumor Ag, MAGE-3. CD4\(^+\) T cell clones generated from PBMC stimulated with DC pulsed with purified MAGE-3 protein recognized peptide or protein pulsed on HLA-DR13-matched EBV B cells, but not MAGE-3\(^+\)/DR13\(^+\) tumor cells (13). However, in another study, CD4\(^+\) T cells generated from PMBC stimulated with peptides predicted by a computer-assisted algorithm were capable of recognizing both peptide pulsed on EBV B cells and MAGE-3\(^+\)/DR13\(^+\) tumor cells (15). In the case of NY-ESO-1, we show in this study that CD4\(^+\) T cells can recognize the NY-ESO-1 protein or peptide pulsed on DR4-matched EBV B cells as well as tumor cells expressing NY-ESO-1 (Figs. 3 and 4). Utilization of HLA-DR transgenic mice may have advantages in identifying putative epitopes because immunized transgenic mice presumably have a high precursor frequency of specifically reactive T cells. Once candidate peptides were identified, CD4\(^+\) T cells could be generated from PBMC stimulated with synthetic candidate peptides. Therefore, the combined use of transgenic mice immunized with the whole protein and stimulated with the peptides predicted by a computer-assisted algorithm may avoid the need to stimulate human PBMC with a large number of peptides and several rounds of in vitro stimulation. Furthermore, candidate peptides identified by using the immunized transgenic mice may be peptides that are naturally processed and presented on the cell surface. This may increase the likelihood that peptide-specific CD4\(^+\) T cells can recognize tumor cells as well. Finally, the use of PBMC from a patient (TE), who developed a high titer of Ab and a high precursor frequency of CTL against NY-ESO-1, may make it easier to generate tumor-specific CD4\(^+\) T cells because both Ab production and CTL require the help of CD4\(^+\) T cells. This approach has been used to identify a number of MHC class II-restricted T cell epitopes from known autoantigens involved in autoimmune disease (29). Therefore, the strategy used in this study may be applicable to many other known MHC class I-restricted tumor Ags, while other strategies such as a
direct gene-cloning approach may facilitate the identification of unknown MHC class II-restricted tumor Ags.

In this study, we observed that ESO p116–135 was recognized by TE4-1 when pulsed on 293IMDR1 cells at a relatively high peptide concentration (Fig. 3B). T cell recognition was significantly reduced when peptide concentrations decreased from 33 to 3.3 μM for the peptide titration experiment (data not shown). In a separate experiment, the peptide ESO p116–135 as well as the whole NY-ESO-1 protein were weakly recognized when pulsed on DR1–586 EBV B cells. The recognition was specifically blocked by anti-DR Ab, but not anti-class I Ab and control Ab (data not shown). TE4-1 cells also only recognized DR1-positive tumor cell line F050 mel expressing a high level of NY-ESO-1, but not DR1-positive1300 mel expressing a low level of NY-ESO-1 (Fig. 4). Thus, this T cell recognition may be explained by two possibilities. The peptide ESO p116–135 may be promiscuous and can be recognized by TE4-1 in the context of either DR1 or DR4, but with different affinities. It has been shown that T cells can degenerately recognize peptides presented by multiple HLA class II alleles (30, 31). Alternatively, ESO p116–135 might bind to DR1 molecule. The carried-over peptide is subsequently released and presented to each other by DR4-positive TE4-1 T cells. T cell-T cell presentation has been reported in CD4+ TIL1363 (17).

Clinical trials using peptides derived from tissue-specific differentiation Ags such as gp100 showed some evidence of therapeutic efficacy in the treatment of patients with melanoma (3). Although no significant toxic side effects were observed in the patients treated with the modified gp100 peptides, vitiligo or depigmentation was often found in patients who responded to therapy (32), suggesting that antitumor immunity induced by immunization with self Ags may cause autoimmunity. In animal studies using TRP-1 as an immune target, similar results (antitumor immunity and coat depigmentation) were also obtained (33–35). Interestingly, antitumor immunity and autoimmunity mediated by gp75/TRP-1 appeared to involve CD4+ T cells and Abs (36). Immunization of mice with human TRP-2 (37), but not murine TRP-2 (38), broke tolerance to the self Ag, and the antitumor immunity required the participation of both CD4+ and CD8+ T cells (36). These studies suggested that antitumor immunity could be mediated by either Abs or CD8+ T cells, but both require the critical help of CD4+ T cells (24, 36).

The MHC class II-restricted NY-ESO-1 peptides identified in this study may be useful in clinical applications because CTL and Abs against NY-ESO-1 were detected in patients with cancer. Immunization with both MHC class I- and II-restricted peptides or
with a purified NY-ESO-1 protein may induce NY-ESO-1-specific CD4+ CD8− T cells as well as Abs. Alternatively, patients could be immunized with dendritic cells loaded with both class I and II peptides or infected with recombinant viruses encoding the NY-ESO-1 gene. Because testicular germ cells do not express MHC class I and II molecules (39), immune responses against NY-ESO-1 should be specific for tumor cells, and thus generate little or no autoimmune responses. Similar studies using MHC class I-restricted peptides of MAGE-3 or peptides pulsed on dendritic cells indicated that while antitumor immunity (CTL responses) and slow tumor regression were demonstrated, no depigmentation/vitiligo or other significant side effects were observed (5, 6). Antitumor immunity may be enhanced by providing tumor-specific CD4+ T cell help.

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References


