The Tumor Antigen Cyclin B1 Hosts Multiple CD4 T Cell Epitopes Differently Recognized by Pre-Existing Naive and Memory Cells in Both Healthy and Cancer Donors

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*J Immunol* 2015; 195:1891-1901; Prepublished online 1 July 2015; doi: 10.4049/jimmunol.1402548
http://www.jimmunol.org/content/195/4/1891

Supplementary Material

http://www.jimmunol.org/content/suppl/2015/07/01/jimmunol.1402548.DCSupplemental

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The Tumor Antigen Cyclin B1 Hosts Multiple CD4 T Cell Epitopes Differently Recognized by Pre-Existing Naive and Memory Cells in Both Healthy and Cancer Donors

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Cyclin B1 (CCNB1) is considered as a potential target for a cancer vaccine, as it is overexpressed in many malignant cells, while being transiently expressed in normal cells. To evaluate the CD4 T cell response to CCNB1, we derived T cell lines by multiple weekly rounds of stimulation with recombinant CCNB1 of T cells collected in healthy donors (long-term T cell assays). T cell lines were specific for 15 immunodominant peptides and derived preferentially from naive T cells. From 74 overlapping peptides, 20 peptides were selected for their broad specificity of binding to HLA class II molecules and included most of the immunodominant epitopes. They primed in vitro a large number of specific CD4 T cell lines in all the donors. Immunodominant epitopes were the most efficacious in long-term T cell assays, both in terms of number of specific T cell lines and number of responding donors. The 20 peptides were also submitted to short-term T cell assays using cells collected in healthy and cancer patients with the aim to evaluate the memory response. The recognized peptides differed from the immunodominant peptides and were part of the best promiscuous peptides. We also observed pre-existing CCNB1-specific IgG Abs in both healthy and cancer donors. Long- and short-term T cell assays revealed that CCNB1 contained many CD4 T cell epitopes, which are differentially recognized by pre-existing naive and memory CD4 T cells. These observations are of value for the design of cancer vaccines. The Journal of Immunology, 2015, 195: 1891–1901.

Tumor-specific CD4 Th lymphocytes are known to enhance and sustain the tumor-specific CD8 T cell and Ab response by providing cytokines and costimulatory signals (1). They also help limit tumor expansion by recruiting and activating phagocytes, by secreting inflammatory cytokines, or by exhibiting direct cytolytic functions (1). Mainly demonstrated in animal models, helper functions of CD4 T lymphocytes have also been confirmed in vaccine trials and associated with clinical benefits (2). CD4 T lymphocytes recognize tumor Ags as antigenic peptides presented by HLA class II heterodimers. Because of the polymorphism of HLA class II molecules, CD4 T cell epitopes are variable in humans. During the last decades, many tumor Ags have been shown to elicit CD4 T cell responses and many T CD4 cell epitopes have been identified (3–8). Besides differences in their expression in normal and tumor tissues and in their level of dispensability, tumor Ags also differ in their ability to mount spontaneous specific CD4 T cell responses in cancer patients. Ags such as those discovered by the SEREX approach (9), but not only these (10), could generate a spontaneous Ab response in cancer patients, sustained by a spontaneous CD4 T cell response (11, 12). Other Ags such as MAGE Ags rarely do so (13). Lack of spontaneous CD4 T cell responses does not preclude the capacity to prime immune responses, but suggests that pre-existing CD4 T lymphocytes in cancer patients are not memory, but naive cells. In contrast, pre-existing CD4 T lymphocytes involved in spontaneous responses might be expanded by the tumor Ag and skewed to different phenotypes varying from effector to exhausted T cells (14, 15). Already identified CD4 T cell epitopes in tumor Ags exhibit different immunological properties. Although some CD4 T cell epitopes are restricted to particular, albeit frequent, HLA class II alleles (4, 5), others are promiscuous and immunogenic in multiple donors (6–8). They also differ by the amplitude of the T cell response they generate. Some tumor-specific T cell epitopes recruit many CD4 T lymphocytes after their injection, whereas others elicit a modest response only (16, 17). In other antigenic systems, this discrepancy has been found to be related to the number of pre-existing CD4 T lymphocytes. In mice, the size of

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Received for publication October 6, 2014. Accepted for publication June 3, 2015.

This work was supported by the Laboratoire d’Excellence en Recherche sur le Médicament et l’Innovation Thérapeutique, Laboratoire d’Excellence Institut de Recherche Vaccinal (to B.M.), the Laboratoire d’Excellence Immuno-Oncologie (to E.T.), Sites de Recherche Intégrés sur le Cancer, Cancer Research for Personalized Medicine (to E.T.), the Ligue contre le Cancer (to E.T.), and the Commissariat à l’Energie Atomique (to B.M.).

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The online version of this article contains supplemental material.

Abbreviations used in this article: CCNB1, cyclin B1; DC, dendritic cell; iDC, immature DC; KLH, keyhole limpet hemocyanin; rGM-CSF, human rGM-CSF; rHLA-A, human rHLA-A; rHLA-B, human rHLA-B; rHLA-C, human rHLA-C; rHLA-D, human rHLA-D; Treg, T regulatory.

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www.jimmunol.org/cgi/doi/10.4049/jimmunol.1402548

The Journal of Immunology

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the naive CD4 T cell repertoire from various T cell epitopes was correlated with the amplitude of the T cell response (18), whereas in humans the frequencies of epitope-specific memory CD4 T cells in vaccines were directly correlated with the frequencies of precursors in the naive repertoire (19, 20). In fact, little work has been done in multiple donors on the size of the repertoire of CD4 T cells specific for epitopes identified in tumor Ags (16, 17, 21). As a result, their in vivo helper activity could be limited by the responder frequency and the number of recruited CD4 T lymphocytes. Moreover, the efficacy of immune checkpoint blockers may be influenced by the naive or activated status of the tumor-infiltrating T cells, as activated T cells can express checkpoint inhibitors (15, 22), unlike naive cells. Together these properties are expected to impact vaccine efficacy considerably, and therefore need to be carefully investigated and probably revisited for tumor Ags discovered early.

Cyclin B1 (CCNB1) regulates the transition from G2 to M phase of the cell cycle and is therefore required for the cell to enter mitosis. CCNB1 is expressed at an almost undetectable level in normal cells, but is overexpressed in many tumors at high constitutive levels. Overexpression has been observed in several human solid tumors, including breast (23), lung (24), colon (25), prostate (26), and head and neck (26) cancers. Tumor-specific CTL response raised against CCNB1 has scarcely been investigated and only in healthy donors (30). Strikingly, it has also been observed that CCNB1-specific Abs pre-exist in healthy donors (30, 31). Because CCNB1 is widely expressed in malignancies and is not dispensable for tumor cells, we evaluated a part of its vaccine only in healthy donors (30). Strikingly, it has also been observed that CCNB1-specific Abs pre-exist in healthy donors (30, 31).

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Materials and Methods

Peptides

CCNB1 peptides were from Activotec, Intavis, or Pepscan or were synthesized using standard 9-fluorenylmethoxycarbonyl chemistry on an Advanced Chemtech synthesizer (Advanced ChemTech Europe), cleaved from the resin by 95% trifluoroacetic acid, and purified by reverse-phase HPLC on a C18 Vydac column (Interchim). The sequence of each peptide was assessed by mass spectrometry. Sequences of overlapping 15-mer CCNB1 peptides were optimized for binding to HLA class II molecules, that is, chosen so that they contained aliphatic or aromatic residues in the N-terminal part of the peptide, a requisite for binding to HLA-DR molecules (32). The peptides encompass the whole CCNB1 sequence. Biotinylated peptides were also synthesized using the standard 9-fluorenylmethoxycarbonyl strategy, as described above. Biotin was introduced using biotinyl-6-aminocaproic acid (Fluka Chimie) on the N-terminal part of the peptide, a requisite for binding to HLA-DR molecules (32). The peptides encompass the whole CCNB1 sequence. Biotinylated peptides were also synthesized using the standard 9-fluorenylmethoxycarbonyl strategy, as described above. Biotin was introduced using biotinyl-6-aminocaproic acid (Fluka Chimie) on the N-terminal part of the peptide, a requisite for binding to HLA-DR molecules (32).

Proteins

Recombinant his-tagged human CCNB1 (Uniprot P14635) was expressed in BL21 Escherichia coli using pET28a (Novagen) transfected by electroporation (2500 V, 2.8 ms) or heat shock. Protein expression was induced by incubation with 1 mM isopropyl-β-D-thiogalactoside (Eubio) at 15°C or 20°C for 21 h. Cell extract was produced by mechanical cell lysis using a cell disrupter (Constant Cell Disruption System). Proteolytic degradation was limited by addition of PMSF (Sigma-Aldrich) or EDTA-free complete protease inhibitor mixture (Roche). DNA removal was performed using benzonase (Invitrogen) in the presence of MgCl2. Clear cell extract was loaded on a HisTrap column (GE Healthcare), and His-tagged recombinant CCNB1 was eluted in an imidazole gradient monitored by an AKTA chromatography system (GE Healthcare).

Only fractions homogeneous on SDS-PAGE were retained. Alternatively, the pooled fractions were further purified using a Superdex 75 column (GE Healthcare). The homogeneity of each batch was assessed by MALDI-TOF mass spectrometry after trypsin degradation. Survivin (Bir5) (Uniprot O15392) was produced in Escherichia coli as fusion proteins with the GST protein, purified on a glutathione column, and separated from GST by proteolytic cleavage. Recombinant his-tagged human DoC (Uniprot P0AEG6) was expressed in BL21 Star E. coli with a plasmid derived from pUC82KA (EMBL) and purified using a HisTrap HP 5 ml column (GE Healthcare). Keyhole limpet hemocyanin (KLH) was from Thermo Fisher Scientific.

Peptide-binding assays specific for HLA class II molecules

Homogenous EBV cell lines were used as sources of HLA class II molecules, as previously reported (8). HLA-DR and HLA-DP molecules were immunopurified by affinity chromatography using monomorphic mAbs L243 and B7/21, respectively. Binding properties of the 75 overlapping 15-mer CCNB1 peptides were determined by competitive ELISA, as previously described (8). Sequences of the biotinylated reporter peptides used in each assay and IC50 values of their unlabeled forms (reference peptides) were the following: hemagglutinin 306-318 (PKYYVKQNLTKL) for DRB1*0101 (4 nM), DRB1*0401 (33 nM), DRB1*1101 (10 nM), and DRB5*0101 (8 nM); YKL (AAAYAAKAAALAA) for DRB7*0101 (16 nM); A3 152-166 (EAEQLRAYLDGTGVE) for DRB1*1501 (40 nM); MT 2-16 (AKTAYDEEEARGGL) for DRB1*0301 (340 nM); B1 21-36 (TERYLVTVRHYNRE) for DRB1*1301 (600 nM); LOL 191-210 (ESWGVAVRDPDKLTGPT) for DRB3*0101 (16 nM); E2/E168 (AGDLAIAETDKAT) for DRB4*0101 (24 nM); and Oxy 271-287 (EKKYFAATQFEPLAALR) for DPB1*0401 (15 nM) and HLADPB1*0402 (25 nM). Data were expressed as relative affinity obtained by dividing the IC50 of the peptide by that of the reference peptide.

Blood samples and HLA-DR genotyping

 Buffy coats from healthy individuals were provided by the Etablissement Français du Sang (Rungis, France). They were collected from anonymous donors after informed consent following the Etablissement Français du Sang guidelines. Blood samples from 14 cancer patients (5 head and neck cancers and 9 lung cancers) and 4 healthy donors were collected at the Hôpital Européen Georges Pompidou (Paris, France) in accordance with French law and after approval by the local ethics committee (CPP Ile de France n°2013-06-03). Patients had not received any cancer treatment yet, and were not treated by immunosuppressive drugs or for specific diseases. PBMCs were isolated by Ficoll-Paque PLUS density gradient centrifugation (GE Healthcare). The HLA-DR genotypes were determined using the Gold SSP DRB1 typing kit (Invitrogen) after DNA extraction from PBMCs with NucleoSpin Blood L. Kit (Macherey Nagel).

Generation and Ag-specific T cell lines from healthy donors

Monoocyte-derived dendritic cells (DCs) were generated from plastic-adherent cells of PBMCs after 4 or 7 d of culture in AIM-V medium (Invitrogen) supplemented with 1000 U/ml human rIL-4 (rhIL-4) and human rGM-CSF (rhGM-CSF; both from R&D Systems). Immature DCs (iDCs) were harvested at day 4 or 5, whereas mature DCs were collected after further 2 d of culture with 1 µg/ml LPS (Sigma-Aldrich). iDCs were loaded with either CCNB1 (1.2 µM) or KLH (0.25-1.2 µM) diluted in AIM-V medium supplemented with 1000 U/ml rhIL-4, 1000 U/ml rhGM-CSF, and 1 µg/ml LPS and incubated overnight at 37°C. Mature DCs were mixed with a mixture of CCNB1 peptides (each at 1 nM) and incubated at 37°C for 4 h. At 37°C, CD4+ T cells were isolated from autologous non-adherent PBMCs by positive selection using magnetic labeling with anti-CD4 mAbs conjugated to magnetic microbeads, followed by magnetic cell sorting, as recommended by the manufacturer (Miltenyi Biotec). For some experiments, CD4+CD45RA+ and CD4+CD45RO+ were isolated by negative selection using the Naive CD4+ T Cell Isolation Kit II and the Memory CD4+ T Cell Isolation Kit (Miltenyi Biotec). Cell sorting was performed by the manufacturer. Purity of the purified subset was at least >95%. A total of 1×105 protein- or peptide-loaded DCs was added to 1×105 autologous CD4+ T cells seeded in round-bottom wells of culture plates in 200 µL IMDM (Invitrogen) supplemented with 10% human AB serum (Lonza), 0.24 mM glutamine, 0.55 mM ascoragine, 1.5 mM arginine (all amino acids from Sigma-Aldrich), 50 U/ml penicillin, and 50 µg/ml streptomycin (Invitrogen) in the presence of 1000 U/ml rhIL-4 and 10 ng/ml IL-12 (both R&D Systems). CD4+ T lymphocytes were restimulated on days 7 and 14 with 1×105 autologous DCs freshly loaded with a protein or peptides depending on the donor, and were grown
in complete IMDM supplemented with 10% FCS, IL-2 (R&D Systems) and 5 ng/mL IL-7 (R&D Systems). An additional stimulation at day 21 was needed in the case of generation of CCNB1 peptide-specific CD4+ T cell lines. The specificity of the CD4+ T cell lines was assessed at least 5 days after the last stimulation by IFN-γ ELISPOT, as described below.

**Evaluation of T cell line specificity by IFN-γ ELISPOT**

Multiscreen hemagglutinin 96-well plates (Merck Millipore) were coated overnight at 4°C with 2.5 µg/ml anti-human IFN-γ mAb (1-D1K; Mabtech) in PBS (Invitrogen). Wells were saturated for 2 hours at 37°C with complete IMDM and washed with PBS. Proteins (3 µM) were loaded onto iDCs in IMDM supplemented with 1000 U/ml rhIL-4 and 1000 U/ml rhGM-CSF for 4 hours at 37°C, whereas peptides (10 µg/ml) were directly added to Multiscreen plates. PBMCs (5 x 10^5/well) or iDCs (5 x 10^5/well) or lymphoblastoid cell lines (5 x 10^7/well) were used as APCs and cocultured in the plates with ~5-15 x 10^4 CD4+ T cells in 10% FCS supplemented with 0.5 ng/ml IL-7. After overnight incubation at 37°C and washing, plates were subsequently treated with 0.25 µg/ml biotinylated anti-human IFN-γ mAb (7-B6-1; Mabtech) in PBS/1% BSA, extravidin-phosphate (dilution 1:3000 in PBS/0.05% Tween 20/1% BSA; Sigma-Aldrich), and NBT/5-bromo-4-chloro-3-indolyl phosphate (Sigma-Aldrich).

Spot number was determined by the AID ELISPOT Reader System. CD4+ T cell lines were considered as specific when a spot count was 2-fold higher in the presence of the protein or peptide than in its absence, with a minimal difference of 10 spot-forming cells/10^{5} cells (34). A response was considered positive when the number of specific T cells expressed as spot-forming cells/10^{5} cells was calculated after subtracting negative control values (background).

**Assessment of CCNB1-specific CD4+ T cell responses by FluoroSpot assay in cancer patients**

The FluoroSpot assay was described previously (33, 34). Briefly, PBMCs were cultured for 7 days at 2 x 10^5 cells/ml in 2 ml/well pool of CCNB1 peptides (individual peptides at a concentration of 5 µg/ml) complete RPMI/10% FCS medium supplemented with 5% serum AB. On day 1 or 2, IL-2 (Chiron) was added at 50 IU/ml. Peptide specificity of the T cells was assessed at day 7 by means of the IFN-γ-IL-5 FluoroSpot assay (CTL, Bonn, Germany). Briefly, 96-well polyvinylidene difluoride plates (Millipore) were coated and saturated at the same time with 80 µL/well capture anti-human IFN-γ and IL-5 mAb (CTL) and incubated overnight at 4°C. Cultured T cells were added with the individual peptides (at a concentration of 10 µg/ml) to triplicate wells at 10^5 cells/well in AIM V medium for 24 h at 37°C in 5% CO_2. After washing, 80 µL detection solution (fluorescent labeled anti-IFN-γ and IL-5 detection Ab) was added to each well for 120 min at room temperature. After washing, 80 µL amplifying fluorescent solution with Alexa Fluor 488 (visualizes IFN-γ) and CTL Red (visualizes IL-5) was added to each well for 60 min at room temperature. Spots were counted using an automated ELISPOT Reader System (CTL).

The number of specific T cells expressed as spot-forming cells/10^5 cells was calculated after subtracting negative control values (background). Cells incubated with medium alone or PMA (100 ng/ml; Sigma-Aldrich) were used as negative and positive controls, respectively. A response was considered positive when the spot count was 2-fold higher in the presence of the peptide than in its absence, with a minimal difference of 10 spot-forming cells/10^5 cells (34).

**Anti-CCNB1 Ab responses in healthy individuals and cancer patients**

Plasma from 104 healthy individuals was collected from blood samples provided by the Etablissement Français du Sang. Plasma from 54 cancer patients and 12 healthy donors was collected at the Hôpital Européen Georges Pompidou. Wells of Nunc Maxisorp 96-well plates (Thermo Scientific) were coated for 1 hour at 37°C with 0.25 µg/ml human CCNB1 or DTC in 1× PBS (100 µL/well), or with 100 µL/well 1× PBS alone (negative control wells). After being emptied, all the wells were saturated overnight at 4°C with 250 µL/well saturating buffer (1× PBS/0.5% BSA/0.03% Thimerosal; Sigma-Aldrich). Plates were washed four times with 1× PBS/0.05% Tween 20 (Sigma-Aldrich) before incubation overnight at 37°C with 100 µL/well plasma sample diluted (1:400) in dilution buffer (PBS/0.2% BSA/0.05% Tween 20). Plates were subsequently washed and treated with 100 µL/well peroxidase-conjugated goat anti-human IgG (Jackson ImmunoResearch Laboratories; dilution 1:50,000) in dilution buffer for 1 hour at 37°C before addition of 100 µL/well 3,3’5,5’-tetramethylbenzidine (Sigma-Aldrich). After 10-min incubation, the reaction was stopped with 25 µL/well 1 M H_2SO_4 and absorbance was read at 450 nm using a Victor III spectrophotometer (Perkin Elmer). Results were expressed as the difference between the mean of duplicate wells of A_450 values and A_100 values obtained with BSA-saturated uncoated duplicates.

**Results**

**In vitro CD4 T cell response to CCNB1 is supported by multiple CD4 T cell epitopes**

To identify immunodominant CD4 T cell epitopes of CCNB1, we derived CD4 T cell lines specific for CCNB1 from eight healthy donors with diverse HLA class II molecules by a long-term T cell assay. Purified CD4+ T lymphocytes were seeded in multiple wells and stimulated weekly by autologous DCs previously loaded with KLH or CCNB1 to enrich the cocultures in Ag-specific CD4+ T lymphocytes. After three rounds of stimulation, each independent T cell line (CD4 T cells present in a single well) was evaluated for its Ag specificity by IFN-γ ELISPOT. Almost all the T cell lines raised against KLH introduced as positive control were stimulated with DCs loaded with KLH and were therefore specific for KLH (data not shown). T cell specificity to CCNB1 was evaluated using overlapping peptides encompassing the whole sequence of CCNB1 in two subsequent ELISPOT assays. The first ELISPOT experiment was performed against 74 overlapping peptides encompassing the whole sequence of CCNB1 and dispensed into 7 pools. This ELISPOT was followed by a second ELISPOT assay to confirm the specificity of the positive T cell lines and to identify the antigenic peptides present in the stimulating peptide pool.
peptide pool (Fig. 1A). As an example, T cell line 803.6 from donor 803 was specific for the peptide 1–15, whereas another T cell line (803.12) from the same donor was specific for two overlapping peptides, namely 374–388 and 377–391. T cell lines from donors 693 (693.17 and 693.24) and 784 (784.25 and 784.29) were specific for the peptides 280–294 and 419–433, respectively. All donors generated a T cell response specific to CCNB1. Because of the generally low frequency of Ag-specific T cells in healthy donors, an average of 17% of the T cell lines contained CCNB1-specific CD4 T lymphocytes. By considering the proportion of Ag-specific T cell lines among wells seeded at the initiation of the culture, we calculated estimated frequencies of pre-existing Ag-specific CD4 T cells in the blood of the donors, as previously described (20, 35) (Fig. 1B). Although a mean of 9.2 pre-existing T cells per million CD4 cells was found for KLH, a mean of 0.8 cells was observed for CCNB1. This response was supported by 15 different immunodominant peptides (Fig. 1C) as they participate in the CCNB1-specific T cell response. For four peptides (1–15, 285–299, 410–424, and 419–433), multiple specific T cell lines were derived, peptides 1–15, 285–299, and 410–424 being common to different donors. We also investigated the CD4 T cell subsets, from which originated the T cell response to CCNB1. We isolated from five supplementary donors CD4+CD45RO+ and CD4+CD45RA+ populations and amplified them by weekly rounds of antigenic stimulation with recombinant CCNB1 (Fig. 2). Approximately one peptide-specific CD4 cell per million CD4 cells was found for the CD45RA cells, whereas it was close to 0.5 for the CD45RO cells. As it was unlikely that CCNB1-specific T cell lines derived from highly differentiated CD45RA CD4 T cells that weakly proliferated in vitro (36), we concluded that CCNB1-specific T cell lines preferentially originated from the CD45RA naive cells and to lesser extent from CD45RO memory cells.

**Binding of CCNB1 peptides to purified HLA class II molecules**

To shed further light on T cell reactivity to CCNB1, the 74 CCNB1 peptides comprising the full protein sequence were tested for binding to 12 common alleles of HLA-DR or DP (Fig. 3). These alleles comprise 7 molecules encoded by the HLA-DRB1 gene, 3 second HLA-DR molecules, and 2 HLA-DP4. They are all present at a minimum frequency of 5% in the Caucasian population. Many CCNB1 peptides bound to a large panel of HLA class II molecules, including molecules encoded by the HLA-DRB1 gene, which are the main molecules involved in T cell response (shaded bars). Immunodominant T cell epitopes that we previously identified (bold), such as 285–299, 320–334, and 323–337, exhibited

**FIGURE 2.** Induction of CCNB1-specific T cell lines with CD45RA or CD45RO cells. CD45RA and CD45RO cells from five healthy donors were purified by negative selection using immunomagnetic beads and cultured with autologous DCs loaded with recombinant CCNB1. After 3 wk, CD4+ T cell specificity was analyzed by IFN-γ ELISPOT assays using the library of overlapping peptides. The difference between the two conditions was significant in the paired Student t test (p < 0.05).

**FIGURE 3.** Capacity of binding to HLA class II molecules of overlapping CCNB1 peptides. Seventy-five overlapping 15-mer peptides covering the sequence of human CCNB1 were submitted to competitive ELISA specific for 12 HLA class II molecules. Binding activities were expressed as relative activity (ratio of the IC50 of the peptide to the IC50 of the reference peptide, which is a high binder to the HLA-DR molecule). Means calculated from at least two independent experiments. Relative affinities ≥100 correspond to good affinities and were used to count the good binders to HLA-DRB1–encoded molecules (shaded) and to second HLA-DR (DRB3, DRB4, and DRB5) and -DP molecules (blank). Sequences of immunodominant peptides are in bold. Peptides selected for further studies included immunodominant peptides (black arrows) and the others (white arrows).
an extremely broad specificity for HLA class II molecules as they bound with high affinity up to 12 molecules. Many other immunodominant epitopes bound to at least half of the HLA class II molecules, peptide 315–329 being active for 2 molecules only. It was noteworthy that a large set of nonimmunodominant peptides bound to multiple HLA class II molecules. This was especially the case for peptides between the 199 and 264 positions of the CCNB1 sequence, which tightly bound between 8 and 12 HLA class II molecules. Peptide 363–377 was also found to bind 11 molecules. On the basis of their binding properties, 27 peptides were retained for further studies (Fig. 3, Supplemental Table I). Eleven immunodominant peptides (black arrows) were selected as they bound to at least 4 molecules. Sixteen other peptides were also included as they are part of the peptides that bound to at least half of the HLA class II molecules (white arrows). As some of these peptides were overlapping, the 27 peptides were finally combined into 20 different peptides.

The selected peptides induce multiple peptide-specific CD4 T cell lines in HLA-unrelated healthy donors

Thirteen healthy donors with diverse HLA-DR typing were selected for these experiments (Table I). Mature DCs were loaded with each of two pools of 10 peptides and cocultured with purified CD4 T lymphocytes. After four weekly rounds of stimulation with loaded DCs, the peptide specificity of the T cell lines was assessed by two subsequent ELISPOT assays. As illustrated by the high number of 203 specific T cell lines identified, the T cell response specific to the 20 selected peptides was of strong amplitude and multiepitopic, all the donors being responders (Table I). The average number of T cell lines was 16 per donor. On the basis of the number of specific T cell lines, an average of two CD4 T cell lines specific for the CCNB1 peptides per million CD4 T cells was estimated to pre-exist in the blood of the donors (Fig. 4A). All of the peptides generated T cell lines, but with different yields. Immunodominant T cell epitopes 1–15, 280–299, 299–317, 374–388, and 416–433 were the most T cell–stimulating peptides as evaluated by the number of T cell lines or the number of responding donors (Fig. 4B, Table I). Many other non-immunodominant peptides, such as 168–182, 207–221, 216–230, 221–235, 225–239, 241–258, 267–281, and 363–380, generated multiple T cell lines and were found to be active in at least one third of the donors. To evaluate whether corresponding T cell epitopes were also accessible to T cell recognition upon processing of CCNB1, immature DCs loaded with CCNB1 were used as APCs in the ELISPOT assay. T cell lines 23.46, 56.29, 334.9, and 334.7 were stimulated by autologous DCs loaded with recombinant CCNB1, but not with recombinant Survivin (Bir5) or a bacterial His-tagged protein (DsbC) (Fig. 5A). Similar experiments showed specific T cell activation upon DC loading with CCNB1 for five immunodominant epitopes and three non-immunodominant ones, namely 168–182, 199–216, and 225–239 (Fig. 5B). We also assessed the direct recognition of CCNB1-expressing lymphoblastoid HLA-matched cell lines using seven T cell lines obtained from three different donors (829, 876, and 877) (Fig. 5C) and specific to six different peptides. One T cell line specific to the peptide 410–424 was not stimulated by HLA-matched lymphoblastoid cell lines, whereas the six other T cell lines reacted with one HLA-matched lymphoblastoid, but not with other lymphoblastoid cell lines harboring different HLA molecules (Fig. 5C). As a result, recognition of processed CCNB1 has been demonstrated for 14 peptides of the 20 identified peptides either because they stimulated T cells primed with CCNB1 (Fig. 1) or because the T cells they generated were stimulated by recombinant CCNB1-loaded DCs or because the T cell lines were

### Table I. Peptide specificity of T cell lines in vitro induced with 20 selected CCNB1 peptides

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<tr>
<th>Donor</th>
<th>HLA-DRB1 Genotype</th>
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<th>Total responder frequency (no.)</th>
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CD4 T cell lines were obtained as described in Fig. 3. CD4 T cell lines were considered as specific when a spot count was two-fold higher in the presence of the peptide than in its absence, with a minimal difference of 25 spots. Bold indicates immunodominant epitopes (described in Fig. 1).
and for four of them to a concentration inferior to 0.1 mg/ml. Their response was found in four of five head-and-neck cancer and seven of nine lung cancer patients. The average number of spots per peptide for all the donors is reported in Fig. 6B. The amplitude of the IFN-γ response was greater in lung cancer patients than head-and-neck cancer patients. We also introduced in these experiments samples from four healthy donors. A good IFN-γ response was found and involved the peptides 199–216, 207–221, 212–226, 221–235, 225–239, and 241–258 with an average number of spots per peptide in the range of that observed for head-and-neck donors. We therefore conclude that the spontaneous T cell response to CCNB1 is frequent in both healthy and cancer patients, but focuses on few peptides. This response also occurs in healthy donors, raising the question that the tumors might not have been at the initiation of the response. We also looked for CCNB1-specific T regulatory (Treg) cells by evaluating CD69 expression on Foxp3+ CD4+ T cells upon stimulation with CCNB1 peptides (those eliciting the highest CD4+ T cell response in cancer patients), but we did not show any significant response (data not shown). Neither did we show IL-10 and IL-17 secretion in the supernatants of PBMC activated by CCNB1 peptides (data not shown). We finally sorted naive (CD3+CD45RA+CD62L+) and CD3+CD45RO+ memory T cells by cell sorter from the blood of two lung cancer patients (ROUC and HOBS) and evaluated their response to a pool of 9 peptides (199–216, 207–221, 212–226, 221–235, 225–239, 241–258, 342–356, and 363–380) (Fig. 6C). T cell response to the peptide pool was found in both lung cancer patients (ROUC and HOBS) from both naive and memory T cells. For patient ROUC, the memory T cell response to CCNB1 peptides was greater than that generated by naive T cells, whereas the difference is less for patient HOBS. We therefore confirmed that this response observed after 1-wk stimulation emerged from memory cells, but also to a lesser extent from naive T cells.

**Ab response in healthy and cancer patients**

As we found spontaneous T cell responses to CCNB1 in healthy and cancer patients, we evaluated the humoral IgG response to CCNB1 by ELISA (Fig. 7). We first considered a panel of plasma samples from 104 healthy donors and submitted them to ELISA specific for CCNB1 and DsbC, a bacterial his-tagged protein (Fig. 7A). DsbC was produced and purified in a very similar way as CCNB1. Coated on ELISA plates in the same conditions, CCNB1 generated for most of the donors a greater signal than DsbC did. Excess CCNB1 in the wells inhibited the signal produced by a pool of selected plasmas, demonstrating the specificity of the interactions (data not shown). We then evaluated the CCNB1-specific response with plasma from 20 head-and-neck, 15 lung, 14 renal, and 5 other cancer patients and 12 healthy donors (Fig. 7B). All sera generated a greater signal with CCNB1 than with DsbC, but means of anti-CCNB1 IgG response did not significantly differ between the subgroups of donors, including healthy donors. As we investigated both T and B cell responses in 2 healthy and 13 cancer donors, we compared the data obtained in the two studies, but did not find any correlation between ELISA signal and number of spots or number of T cell epitopes (data not shown). We therefore conclude that most of the healthy and cancer peptides possess circulating Abs specific for CCNB1, whose level is unrelated to the cancer status of the patients or the spontaneous CCNB1-specific T cell response.

**Discussion**

Because of its wide expression in tumors and undetectable level in normal cells, CCNB1 is a potential candidate for cancer vaccines, provided it elicits a strong T cell response in multiple donors. We

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**FIGURE 4.** Specificity and magnitude of CD4+ T cell response against CCNB1 peptides in healthy donors. CD4+ T cells from 13 healthy donors were repeatedly stimulated in vitro with two pools of the selected CCNB1 peptides loaded on autologous DCs. CD4+ T cell specificity for the peptide pools and the individual peptides was assessed in two subsequent IFN-γ ELISPOT assays. (A) Estimated frequency of CD4+ T cells specific to CCNB1 peptides, in the blood of healthy donors. (B) Number of specific CD4+ T cell lines (left panel) and frequency of responding donors (right panel) were reported for each peptide. Black bars, immunodominant epitopes.
therefore evaluated the CD4 T cell response specific to CCNB1 in healthy and cancer patients by submitting recombinant CCNB1 and overlapping peptides to different formats of T cell assays and by using binding assays specific for multiple HLA class II molecules. A large pre-existing T cell repertoire in many donors was found to be specific for a multitude of CD4 T cell epitopes that exhibit large discrepancies of recognition by naive and memory cells in both healthy and cancer donors.

We first evaluated the capacity of CCNB1 to prime CD4 T cells in vitro from eight healthy donors with different HLA genotypes to quantify the number of pre-existing CCNB1-specific T cells in healthy individuals and to identify immunodominant peptides (37, 38), that is, peptides contributing to the T cell response to CCNB1. We therefore derived T cell lines by three weekly rounds of stimulation with CCNB1 and assessed the peptide specificity using overlapping peptides encompassing the whole sequence of CCNB1. We have already applied this long-term T cell assay to Ags of various origins (8, 20, 35, 39, 40) and found a very good concordance with their immunogenicity in humans (20, 35, 39).

By this approach, we estimated that there are 0.8 pre-existing CCNB1-specific CD4 cells per million CD4 cells in the blood of healthy donors. All of the donors mounted a CCNB1-specific T cell response. By comparison with tumor Ags that we have already investigated by this approach, this response was qualified as very good (8, 40). This frequency is in the range of that observed for NY-ESO-1 (0.5–5 cells/M), which is considered as one of the prototype tumor Ags for the development of cancer vaccine (21). This frequency is often higher than those observed for individual CD4 T cell epitopes of tumor Ags before vaccination (16, 17, 41). In agreement with these frequencies, we found that T cell lines originated mainly from CD45RA naive cells and to a lesser extent from CD45RO cells. The CCNB1-specific CD4 T cell response was supported by 15 different epitopes that we referred to as immunodominant, 3 of which were common to different donors (1–15, 285–299, 419–424). These epitopes bound between 6 and 11 HLA class II molecules and hence correspond to promiscuous epitopes.

By submitting all of the overlapping CCNB1 peptides to HLA class II–binding assays, we identified multiple peptides with a
broad specificity for HLA class II molecules that were not found to be immunodominant in previous long-term T cell assays. Twenty-seven peptides combined into 20 peptides were selected for further study based on their promiscuity of binding to HLA class II molecules and also included many of the peptides identified in previous T cell assays. All of the peptides gave rise to a T cell response in long-term T cell assays using healthy donors, all of the donors being responders. The number of pre-existing T cells specific for the pools of peptides was higher than that found with the recombinant CCNB1, demonstrating that the T cell repertoire reacting to peptides is larger than that reacting to the whole protein, in agreement with the concept of immunodominance (37). As shown in Fig. 4, immunodominant peptides (1–15, 17–31, 280–299, 299–317, 374–388, 410–424, and 416–433) are among the peptides generating the highest number of T cell lines and having the highest number of responding donors. As this T cell assay relies on the size of the pre-existing repertoire (20, 39, 41), immunodominance of these peptides appears to result from their ability to be recognized by a wide repertoire of CD4 T cells in healthy donors as already proposed for other Ags (19, 39, 42).
These experiments also revealed 12 other peptides with T cell-stimulating properties. Some of them (168–182, 207–221, 221–235, 225–239, 241–258, and 363–380) generated specific T cell lines in at least one third of the donors. We also attempted to evaluate the capacity of the peptide-specific T cell lines to recognize naturally processed peptides of CCNB1 and submitted T cell lines to recognition of DCs loaded with recombinant CCNB1 and HLA-matched CCNB1-expressing cells. Besides previously identified immunodominant epitopes, the peptides 168–182, 199–216, and 225–239 were found in vitro dominant (also called subdominant) (37, 38) as their corresponding T cell lines were stimulated by CCNB1-loaded DCs. As a result, 14 peptides of the 20 identified peptides seemed to be properly generated in APCs either because they have been found to stimulate T cells primed with CCNB1 or because the T cells they generated were stimulated by CCNB1-loaded DC or because these T cell lines were stimulated by matched lymphoblastoid cell lines, but not by others. We were, however, limited in these experiments by the weak solubility and in vitro toxicity of batches of CCNB1 and by the lack of the same cell line expressing and not expressing CCNB1. All transfactable cell lines already express CCNB1, and its expression could not be blocked by gene invalidation probably because CCNB1 is not dispensable for the tumor cells (data not shown). We therefore concluded from these experiments that a large repertoire of CD4 T lymphocytes specific for CCNB1 peptides was revealed in healthy donors after their in vitro amplification in the long-term T cell assay. These cells originate preferentially from naive cells and react to multiple peptides with broad specificity of binding to HLA class II molecules.

The set of 20 selected peptides was also introduced into short-term T cell assays in cancer and healthy donors with the initial aim to evaluate the memory response specific to these peptides. The four healthy donors respond mainly to six peptides, namely 199–216, 207–221, 212–226, 221–235, 225–239, and 241–258. This is clearly reminiscent of a previous study showing that five healthy donors reacted in similar short-term T cell assays to three overlapping peptides (215–229, 219–233, and 223–234) (30). We therefore extended the sequence containing the previously identified peptides (30) with peptides located on both sides of it. As stated above, overlapping peptides of this region of CCNB1 are part of the most promiscuous peptides of CCNB1 as they bound up to 11 different HLA class II molecules. However, in the initial T cell assay, they were not found to be immunodominant. They also did not generate a huge number of T cell lines when pools of peptides were used as immunogens, suggesting that either these T cells are not able to be expanded or that our culture conditions are not appropriate for their expansion. Spontaneous CD4 T cell responses to the cancer Ag OCT4 or MUC-1 have already been observed in healthy donors (43, 44), but remained unusual as compared with other tumor Ags (8, 11, 12, 45, 46). For the first time, to our knowledge, we demonstrated that the spontaneous CD4 T cell response to CCNB1 persists in cancer patients and is not therefore dampened by the tumors. This response appeared to be amplified in lung cancer patients, but did not spread to the other epitopes that we reported above. As a result, only some of the pre-existing CD4 cells specific to CCNB1 epitopes participate in a spontaneous response revealed in both healthy and cancer patients by short-term T cell assays.

The CD4 T cell response to CCNB1 stems therefore from two different populations of CD4 T lymphocytes revealed by short- and long-term T cell assays. CCNB1-specific T cells revealed by the short-term T cell assay are mainly memory cells, but also originate from naive cells. In contrast, Treg cells do not seem to be part of this response. These CCNB1-specific T cells recognized a limited set of epitopes that we reported above. As a result, only some of the pre-existing peptides of CCNB1 as they bound up to 11 different HLA class II molecules. However, in the initial T cell assays using recombinant CCNB1 after alteration of its expression in the periphery. Infection by CMV (47) or varicella zoster virus (48) has been shown to induce overexpression of CCNB1 in infected cells and may favor initiation of the T cell response. Alternatively, memory CCNB1-specific CD4 T cells could have been primed by cross-reactivity with other Ags (49), as suggested by the detection of memory T cells specific to viruses in donors without any previous exposure (42, 50, 51). The CCNB1 sequence 199–237 is identical in orthologs found in farm animals (sheep, horse, pork) and pets (cat, dog, guinea pig), which could be a source of sensitization by contact or ingestion. Its degree of identity falls to 68% for plants, (cat, dog, guinea pig), which could be a source of sensitization by contact or ingestion. Its degree of identity falls to 68% for plants, 64% for fungi, and 59% for bacteria, which are therefore not evident sources of cross-reactivity. The latter hypothesis might explain why the CCNB1 peptides were not immunodominant in the long-term T cell assays using recombinant CCNB1 as an immunogen. Alternatively, their lack of immunodominance in this assay might be due to the in vivo conversion of naive CD4 T cells specific to these epitopes into cells that could not be easily amplified in vitro. They might be also suppressed by CCNB1-specific Treg cells as already reported for other tumor Ags (52), but we did not reveal the existence of these Treg cells. Nevertheless, these hypotheses remain to be validated, and none of them appears as evident.

The second population of CCNB1-specific T cells was revealed by long-term T cell assays and mainly corresponded to naive cells.
Their peptide specificity is large and comprises at least 20 different peptides, including the previously identified peptides. Although their frequencies are elevated for naïve cells, they are lower than those of cells detected in the short-term T cell assays. To our knowledge, this pattern of recognition of CD4 T cell epitopes appears as a singularity of CCNB1 with regard to other self-proteins and tumor Ags.

We also confirmed the spontaneous CCNB1-specific IgG response observed in healthy and cancer patients (30, 31, 53–55). We observed a similar level of anti-CCNB1 Abs in patients and healthy donors (31). This is at variance with other studies in which the level was either higher (53, 54) or lower (55) in cancer patients than in healthy donors. These discrepancies may result either from cancer indications or from a strong variation between individuals, as revealed in Fig. 7 (30, 31). As mentioned by Vella et al. (30), the tumor Ag MUC-1 could also be the target of Abs in noncancer patients that could have been elicited by infection (56), as formally observed for GM-CSF autoantibodies (57). We cannot completely exclude a link between the anti-CCNB1 humoral response and the memory CCNB1-specific CD4 T cells that we detected in the short-term T cell assays, but there was no correlation between T and B cell responses. Clearly, the origin, consequences, and regulation of pre-existing CCNB1-specific memory T and B cells constitute relevant and challenging issues to be elucidated in healthy and cancer patients.

Tumors seem to upregulate immune checkpoint proteins on tumor-specific T cells and limit their expansion (14, 15). Recent trials with Abs specific to checkpoint blockers have shown a clear clinical benefit (58), probably as a result of reversion of tumor-induced T cell dysfunction (14).

Pre-existing immune response specific to tumor Ags is generally not predictive of good or bad outcomes for the patients. The high incidence of the pre-existing T and B cell response to CCNB1 does not suggest that this pre-existing response is protective. However, recent observations indicate that tumor regression after treatment with therapeutic anti-PD1 requires pre-existing T cells (59), including CD4 T cells (60). Pre-existing immunity to tumor Ags, including CCNB1, might therefore participate to therapeutic effects of immune checkpoint blockers. Pre-existing memory T cells could be already present in healthy donors, as in the case of T cells specific to the central part of CCNB1 (30), or could come from priming by the tumors (14, 15) or by tumor-specific vaccines (61). T cells elicited in these different conditions might differ by their phenotypes with regard to expression of checkpoint proteins and to different degrees of sensitivity to blockade by Abs. Because of its wide frequency in tumors and the large and differentiated T cell response that CCNB1 could generate, CCNB1 might be a relevant model for investigating the influence of T cell specificity on efficacy of immune checkpoint blockers.

Acknowledgments

We thank Daniel Gillet and Sylvain Pichard for advice and technical support, and we also thank the clinical investigation center of Cochin Hospital.

Disclosures

C.C., E. Favry, A.M., F.A.C., and B.M. are coinventors of a pending patent entitled Immunogenic peptides of the cyclin B1 tumour antigen and numbered WO2015001526. The other authors have no conflicts of interest.

References

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