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Brief communication

Antiviral cellular immunity in colorectal cancer patients

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ABSTRACT

Immunotherapy is a growing field of interest in the treatment of malignant diseases, such as colorectal cancer (CRC). The induction or enhancement of T-cell responses against tumor-associated antigens is particularly important in tumor vaccination strategies. Successful immunization relies on an intact immune system. Both chemotherapy and the tumor itself are known to potentially inhibit immune responses. In this study we analyzed T cells directed against antigens of cytomegalovirus (CMV) and influenza virus in 39 HLA-A2–positive CRC patients and 29 HLA-A2–positive healthy donors using the tetramer technology. We found no difference between CRC patients and the healthy control group for either the proportion of samples with detection of virus specific T cells or the magnitude of these specific T cells. Although we cannot draw a firm conclusion on T-cell induction in cancer patients during vaccination therapy, our results show that CRC patients retain their antiviral T cells, suggesting a potential susceptibility to immunotherapy. The quantity of adaptive immunity acquired earlier in life seems not to be affected by the presence of CRC.

1. Introduction

Colorectal cancer (CRC) is a common malignant disease with a high potential for immunologic treatment. CRC patients are able to spontaneously develop antigen-specific T-cell response without prior immunotherapy [1,2]. However, no survival benefit was found for patients with such peripheral tumor-associated antigen (TAA)–directed T-cell responses [3]. In addition, no benefit was found for CRC patients with vaccination therapy aiming at the induction of immune responses [4].

Human beings with an intact immune system develop T-cell responses against common viral infections such as influenza or cytomegalovirus (CMV) during their lifetime. Humoral as well as cellular immune responses against virus-specific antigens are described and measured regularly. A reliable assay to assess the cellular epitope-specific immune state is tetramer staining of virus-specific T-cell responses [5]. Influenza and CMV are two of the most common viral diseases for which human leukocyte antigen (HLA)-A*0201 binding epitopes are described [6–10].

It is unknown why CRC patients do not reject their tumor in the presence of a spontaneous immune response against TAAs or during active specific vaccination. One reason might be an altered cellular immune system. The aim of the present study was to quantitatively analyze the cellular immune state against two common viral infections with major histocompatibility complex (MHC)/peptide tetramer staining. A decreased cellular immune activity against viral infections in CRC patients in comparison to healthy donors might be indicative of an efficacy lack of antitumor T cells in CRC.

2. Subjects and methods

2.1. Patients

After institutional review board approval and informed consent, peripheral blood mononuclear cells (PBMC) from CRC patients at various stages of disease and healthy volunteers were collected and frozen for T-cell analysis. Patients were initially hospitalized for diagnostic or therapeutic reasons. Volunteers were recruited from laboratory and clinical colleagues within our department. All analyses were performed in compliance with the Declaration of Helsinki. Patients and healthy donors were tested serologically for expression of HLA-A2 in our routine HLA laboratory.

2.2. T-cell assay (tetramer staining)

HLA-A2-positive patients and donors were tested for the presence of antigen-specific T cells directed against the HLA-A*0201-associated T-cell epitopes FLU M1 GILGFVFTL (MP58-66) and CMV pp65 (NLVPMVATV) using PE-labeled MHC/peptide tetramers according to the manufacturer's instructions (Beckman Coulter). In addition, we stained the cells with 10 μl anti–CD8-FITC. Analysis was performed on a FACscan cytometer (Becton Dickinson). Tetramer-positive cells were identified in the lymphocyte population by gating and thereafter by selecting events with dual expression of CD8 and specific T-cell receptor (TCR) recognized by the tetramer. The percentages of specific T cells were calculated as percentages of CD8 positive T cells. A specific T-cell response was rated as positive if (a) the tetramer positive population after subtraction of the

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negative control reached at least 0.1% of CD8+ lymphocytes; and (b) the tetramer positive population reached at least twice the number of the negative control. As negative control, iTAg MHC Negative Tetramer was used (Beckman Coulter).

2.3. Statistical and survival analysis

The amount of positive antigen-specific T-cell responses in CRC patients and healthy donors was compared with a 2×2 contingency table using the χ^2 or Fisher's exact test. The magnitude of antigen-specific T-cell responses was compared using the Wilcoxon rank-sum test. Mean values are given with two standard errors. Correlations between T-cell responsiveness and age were calculated using Spearman's correlation coefficient.

The level of significance was 0.05 (two-sided). Commercially available statistical software was used (SPSS for Windows, release 14.0; SPSS Inc., Chicago, IL).

3. Results

3.1. Patient characteristics

Peripheral blood mononuclear cells were collected from 39 HLA-A2–positive patients with CRC and 29 HLA-A2–positive healthy volunteers. Median age was 69 years (range, 38–91 years) in the CRC group and 37.5 years (range, 23–65 years) in the control group. Mean time from diagnosis or operation to blood sampling in CRC patients was 2 weeks (range, 0–25). Five patients with rectal carcinoma had received radiochemotherapy before operation and blood sampling, and two patients with metastatic disease had received prior chemotherapy or immunochemotherapy. Characteristics of the CRC patients are summarized in Table 1.

Table 1
Characteristics of colorectal cancer (CRC) patients

Characteristic	CRC patients, *n* (%)
Histology	
Adenocarcinoma	36 (92)
(partially) mucinous carcinoma	3 (8)
UICC staging	
Stage 1	5 (13)
Stage 2	16 (41)
Stage 3	12 (31)
Stage 4	6 (15)
Location	
Colon	23 (59)
Caecum	7 (30)
Ascending colon	5 (22)
Transverse colon	2 (9)
Descending colon	1 (4)
Sigmoid	8 (35)
Rectum	15 (38)
Unknown	1 (3)
Tumor grade	
Grade 1	1 (3)
Grade 2	19 (49)
Grade 3	12 (31)
Grade missing	7 (18)
Previous treatment	
Radiochemotherapy	5
Immunochemotherapy	1
Chemotherapy	1
No previous treatment	32

UICC, Union Internationale Contre le Cancer (International Union Against Cancer).

3.2. CMV specific T-cells

Of the 39 patients with CRC, 20 had a significant number of T cells directed against CMV (51%), and 12 of 29 healthy volunteers (41%) ($p = 0.47$).

The mean percentage of CMV-specific CD8 cells among samples classified as "positive" was 1.66 ± 1.18 (median 0.75; range, 0.1–10.18) in the CRC group ($n = 20$) and 0.52 ± 0.36 (median 0.2; range, 0.1–1.99) in the control group ($p = 0.14$, Mann-Whitney *U* test) (Figure 1). The magnitude of T-cell responsiveness against CMV in positive samples significantly correlated with age ($r = 0.39$). Among patients classified as positive, patients with Union Internationale Contre le Cancer (UICC)/International Union Against Cancer stage 1 and 2 ($n = 12$) did not have a statistically significant difference in the percentage of CMV-specific CD8 cells compared with patients in stage 3 and 4 ($n = 8$) ($p = 0.85$, Mann-Whitney *U* test). CRC patients with a tumor located in the colon ($n = 12$) did not have a higher percentage of CMV-specific CD8 cells compared with rectal cancer patients ($n = 8$) ($p = 0.91$, Mann-Whitney *U* test). Patients with grade 3 disease ($n = 9$) had a higher frequency of CMV-specific CD8 cells compared with those having grade 2 disease ($n = 8$) (2.84 ± 2.41 vs. 0.61 ± 0.41). However, this difference was not significant using the Mann-Whitney *U* test ($p = 0.14$).

3.3. Influenza-specific T cells

Ten of 39 CRC patients showed a significant number of T cells directed against influenza (26%), and so did five of 28 evaluable healthy volunteers (18%) ($p = 0.38$).

The mean percentage of influenza-specific CD8 cells among samples classified as "positive" was 0.49 ± 0.19 (median, 0.43; range, 0.1–0.94) in the CRC group ($n = 10$) and 0.35 ± 0.23 (median, 0.22; range, 0.13–0.76) in the control group ($n = 5$) ($p = 0.37$, Mann-Whitney *U* test; Figure 1). The magnitude of T-cell responsiveness against influenza virus in positive samples significantly correlated with age ($r = 0.59$). No significant difference in T-cell responsiveness was found for tumor location, UICC stage, or tumor grade.

4. Discussion

We did not find statistically significant differences in the frequency of specific T cells directed against common antigens of CMV and influenza virus between CRC patients and healthy individuals. Both healthy donors and CRC patients showed substantial cellular immune responses against these ubiquitous antigens. This observation suggests that the cellular immune system in CRC patients was intact at the time of analysis.

However, adaptive immune responses against common viral antigens develop over time and have most likely been acquired before cancer development. Therefore, although T-cell responsiveness against CMV and influenza virus seems adequate, induction of an immune response against novel TAAs may still be compromised by a cancer-related immunosuppressive environment or immune escape mechanisms, explaining the ineffectiveness of cancer vaccination [11].

A major flaw in our comparison of CRC patients and healthy volunteers is the great difference in median age. On the one hand, exposure to viruses increases over time explaining the greater frequency of virus-specific T cells in the CRC population. On the other hand, the magnitude of immune responses generally decreases with age, particularly because of a numerical and functional impairment of naïve T cells [12,13]. Although elderly individuals are still able to mount a T-cell response after vaccination, the long-term immune response is compromised [14]. Furthermore, response to different viral antigens may differ especially in the elderly population; for example, several authors have reported greater frequencies of CD8+ T cells specific for CMV compared with those specific for influenza, corresponding with our own observation [15,16]. Contrary to observations of an age-related decrease in immunity, we found a modest positive correlation between advanced age and increased virus-specific T cells. The modest albeit not statistically significant increase of immune responses particularly against CMV in the colorectal cancer patient cohort might thus