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ORIGINAL ARTICLE

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Down-regulation of HLA class II and costimulatory CD86/B7-2 on circulating monocytes from melanoma patients

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Abstract Antigen-presenting cells are crucial for the induction of an antigen-specific antitumoral immune response. Deteriorations in the expression pattern of cell surface molecules important for the presentation of antigens might therefore be indicative of an impaired immune response status in cancer patients. In the present study we investigated the expression of MHC class I and class II molecules, of the costimulatory molecules CD80/B7-1 and CD86/B7-2, of the adhesion molecule CD11c, and of the marker of activation CD71 on $CD14^+$ peripheral blood monocytes (PBMs) from 144 melanoma patients in different stages of disease and 43 healthy controls, by flow cytometric analysis. We found a decreased expression of HLA-DR ($p < 0.0005$), HLA-DQ ($p = 0.006$), HLA-DP ($p < 0.0005$), and CD86/B7-2 ($p = 0.001$) on PBMs from melanoma patients compared with healthy controls, whereas no significant difference could be detected in the expression of HLA class I antigens and CD80/B7-1. This down-regulated expression was associated with disease progression. In contrast, CD71 expression was stage-dependently increased on PBMs from melanoma patients compared with healthy controls ($p = 0.024$). No correlation was found between the PBM surface expression pattern and age, gender, tumor load, and current mode of therapy of the patients. The observed down-regulation of HLA class II and CD86/B7-2 on melanoma patients' PBMs might reflect an ineffective antigen-presenting function contributing to an impaired antigen-specific immune response in these patients.

Keywords Melanoma · Monocytes · HLA class I · HLA class II · CD80/B7-1 · CD86/B7-2 · CD71 · CD11c

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Introduction

Tumor cells' escape from hostage immunosurveillance is known as one of the major mechanisms enabling unrestrained neoplastic cell growth and formation of metastases. This immune escape is thought to be supported either by mechanisms of defense exerted by the tumor cells themselves and/or by an impaired function of the host immune system [5]. Numerous therapeutic efforts have been undertaken to restore this defective function, mostly with the intent of strengthening the antigen-specific antitumoral immune response [16, 24]. Since professional antigen-presenting cells (APCs) are known as one of the most important inducers of an antigen-specific immune response, their potentially defective function causes a strong impairment of immunosurveillance in tumor-bearing hosts [17]. Professional APCs include dendritic cells, B cells, and macrophages. Two of these cell types might originate from monocytes circulating in the peripheral blood (PBMs): macrophages by extravasal migration in vivo as well as dendritic cells in vitro cultured with additional cytokines [2]. While dendritic cells are the most effective APCs in the induction of primary immune responses and are considered the best vehicle for the delivery of tumor-associated antigens in the immunotherapy of cancer patients, macrophages play an important role in the immune defense against bacterial and viral infections as well as against tumor cells. Their progenitors, the PBMs,

have been shown to exert a so-called monocyte-mediated tumoricidal activity [7, 18]. This direct cytotoxic effect requires a monocyte-to-tumor-cell contact and is independent of MHC class I or II expression or presentation of tumor-associated antigenic peptides [8, 13]. However, little is known about the potency of PBMs to sufficiently induce an antigen-specific immune response. While broadly expressing the MHC class II cell surface molecules HLA-DR, HLA-DQ, and HLA-DP, PBMs show only low surface expression of costimulatory molecules (e.g., CD80/B7-1 and CD86/B7-2). Nevertheless, cell surface expression of HLA-DR has been shown to be down-regulated on PBMs of polytraumatic patients developing a severe sepsis compared with patients with nonsepticemic outcome [6]. The authors describe HLA-DR expression on PBMs as an indicator of an impaired immune response against bacterial antigens, accompanied by reduced serum concentrations of soluble HLA-DR (sHLA-DR). Confirming this observation, the concentration of HLA-DR in serum has been reported before to reflect a state of immune activation, thus found to be elevated in patients with viral infections, in rejection episodes following organ transplantation, and in graft-versus-host disease [9].

Recently, we found reduced serum levels of sHLA-DR correlated with advanced stages of disease and tumor burden in serum from malignant melanoma patients, associated with poor overall, and low rates of progression-free, survival [23]. These results suggest a corresponding reduction of the cell surface expression of HLA-DR on PBMs from melanoma patients. This possibly impaired HLA class II–expression pattern might reflect a defective immune response function in these patients leading to a failure in antigen-specific recognition and defense against melanoma cells. In the present study we therefore investigated the expression of HLA-DR and further HLA class II molecules on PBMs from melanoma patients in different stages of the disease compared with healthy controls. Moreover, we analyzed the cell surface expression of HLA class I molecules, of the costimulatory molecules CD80/B7-1 and CD86/B7-2, of the adhesion molecule CD11c, and of the marker of activation CD71.

Materials and methods

Patients

After informed consent was obtained, blood was drawn from 144 unselected patients presenting at the Department of Dermatology, the Saarland University Hospital, Homburg/Saar, Germany, with histologically confirmed malignant melanoma of different stages of disease, and from 43 healthy controls matched in age and gender. Clinical staging of the patients was performed according to the guidelines of the American Joint Committee on Cancer (AJCC) [1]. The patients included 23 males with a mean age of 50.4 ± 16.8 years and 28 females with a mean age of 50.2 ± 15.9 years in stage I (localized melanoma, tumor thickness ≤ 1.5 mm), 21 males with a mean age of 61.2 ± 17.7 years and 18 females with a mean age of 59.2 ± 10.6 years in stage II (localized melanoma, tumor thickness > 1.5 mm), 14 males with a mean age of 58.4 ± 13.3 years and 14 females with a mean age of 63.6 ± 10.2 years in stage III (regional lymph node and/or in-transit metastases) and 12 males with a mean age of 58.9 ± 16.4 years and 14 females with a mean age of 54.8 ± 15.8 years in stage IV (distant metastases). Detailed clinical characteristics of the patients are presented in Table 1. The 43 healthy persons analyzed as controls comprised 23 males with a mean age of 52.6 ± 20.3 years and 20 females with a mean age of 54.1 ± 17.9 years. Patients were enrolled into this study from January until August 2000. Following the results of diagnostic methods such as physical examination, X-ray or CT of the chest, ultrasound or CT of the abdomen as well as MRI of the brain, all patients were classified as tumor-bearing or tumor-free. Patients were treated according to therapy protocols of the Dermatologic Cooperative Oncology Group (DeCOG) including cytostatic (cisplatin, dacarbazine, temozolomide, vindesine) and immunomodulatory (interferon-α) agents in different combinations and schedules. Blood samples of healthy controls were kindly provided by the Department of Hematology and Blood Transfusion of the Saarland University Hospital. All controls were blood donors undergoing regular physical and laboratory examinations.

Monoclonal antibodies (mAbs)

MAbs recognizing HLA-DR (L243) and CD14 (MφP9) were purchased from Becton Dickinson (San Jose, CA, USA), as well as the unspecific antihuman mAb X40. The anti-pan-HLA class I mAb W6/32, the anti-CD71 mAb Ber-T9 and the fluorescein isothiocyanate (FITC)-conjugated rabbit antimouse $F(ab)_2$ fragments were obtained from DAKO (Glostrup, Denmark). MAbs recognizing HLA-DQ (TÜ169), HLA-DP (HI43), CD11c (B-ly6), and CD86/B7-2 (2331) were purchased from PharMingen (San Diego, CA, USA). The anti-CD80/B7-1 mAb 104 was purchased from Immunotech (Marseille, France).

Flow cytometry

Peripheral blood mononuclear cells (PBMCs) were isolated from freshly obtained heparinized blood samples by Ficoll-Hypaque density gradient centrifugation. After 60 min of incubation at 4°C with unspecific rabbit IgG (5 μg/ml PBS) in order to block non-specific Fc-receptor binding on monocytes, cells were incubated for 20 min at 4°C with different mAbs in PBS / 0.5% bovine serum albumin (BSA) / 0.02% sodium azide. Used concentrations of mAbs were 0.5 ng/ml (L243), 2 ng/ml (TÜ 169), 4 ng/ml (B-ly6), 2 ng/ml (HI43), 5 ng/ml (2331), 3.5 ng/ml (W6/32), 3.5 ng/ml (Ber-T9), and 4 ng/ml (104). Unspecific mouse IgG was used as control. After two washings, cells were incubated for 20 min at 4°C with FITC-conjugated rabbit antimouse antibodies (1 μg/ml PBS / 0.5% BSA / 0.02% sodium azide), followed by an incubation with the phycoerythrin (PE)-conjugated mAb MφP9 (1 ng/ml). Stained samples were analysed using a Coulter Epics XL 2 cytometer with the system II software (Coulter Electronics, Miami, FL, USA). Peripheral blood lymphocytes (PBLs) and PBMs were distinguished by forward and side light scatter properties. $CD14^+$ cells were gated as the cell population of interest. These cells were analyzed for their expression of surface markers by comparison of their fluorescence after staining with specific mAb versus staining with unspecific isotype-matched mAb. Results are presented as mean fluorescence intensity (MSFI), which represents the ratio of the mean fluorescence intensities of the specific mAb staining and the irrelevant control Ab staining. A MSFI exceeding 1.20 was scored as a positive staining.

Statistical analysis

Mann-Whitney *U*-test (gender and tumor load), Bonferroni post hoc test (therapy vs no therapy), ANOVA analysis (patients vs controls), and ANOVA regression analysis (stage of disease) were

Table 1 Patient characteristics. *MSFI* (mean specific fluorescence intensity) was determined by flow cytometry and calculated as the ratio of the MFI achieved with specific antibodies/isotype-matched control antibodies. MSFI exceeding 1.20 counted as positive staining. Data represent mean ± SEM or median and percentiles (%/%)

	No. of cases	HLA-I (MSFI)	HLA-DR (MSFI)	HLA-DQ (MSFI)	HLA-DP (MSFI)	CD80/B7-1 (MSFI)	CD86/B7-2 (MSFI)	CD11c (MSFI)	CD71 (MSFI)
Patients	144	29.71 (18.29/39.94)	34.42 ± 12.90***	2.25 (1.71/2.79)*	6.29 ± 3.75***	1.43 (1.14/1.63)	2.02 (1.60/2.42)**	3.27 ± 1.08	1.45 (1.17/1.79)*
Sex									
Male	69	31.04 (22.60/39.13)	34.77 ± 12.63	2.41 (1.98/2.85)	6.41 ± 2.92	1.44 (1.18/1.63)	1.99 (1.66/2.41)	3.35 ± 1.10	1.40 (1.19/1.85)
Female	75	26.83 (17.60/40.39)	33.71 ± 13.32	2.12 (1.59/2.74)	6.24 ± 4.36	1.39 (1.10/1.66)	2.02 (1.56/2.42)	3.23 ± 1.08	1.34 (1.15/1.78)
Stage									
I	51	29.52 (18.33/39.35)	36.81 ± 12.25***	2.09 (1.68/2.63)	6.54 ± 4.98*	1.39 (1.14/1.58)	2.15 (1.64/2.50)**	3.21 ± 1.10*	1.29 (1.14/1.65)*
II	39	34.49 (23.71/45.55)	33.27 ± 12.33***	2.56 (2.13/3.00)	6.66 ± 2.47*	1.39 (1.17/1.58)	2.04 (1.73/2.45)**	3.47 ± 1.09*	1.34 (1.17/1.72)*
III	28	27.35 (18.25/38.35)	31.28 ± 14.12***	2.20 (1.62/2.87)	5.59 ± 2.89*	1.47 (0.99/2.29)	1.99 (1.55/2.54)**	3.21 ± 1.03*	1.42 (1.23/1.99)*
IV	26	25.46 (17.53/37.51)	34.97 ± 13.48***	1.99 (1.59/2.61)	5.95 ± 3.24*	1.45 (1.15/1.81)	1.96 (1.52/2.30)**	3.10 ± 1.11*	1.55 (1.16/2.26)*
Tumor load									
Detectable	26	24.07 (17.42/34.92)	29.59 ± 13.18	2.04 (1.57/2.57)	5.07 ± 2.59	1.45 (1.10/1.90)	1.86 (1.52/2.18)	2.81 ± 0.97*	1.44 (1.18/1.79)
Not detectable	118	93.79 (66.79/129.25)	35.32 ± 12.76	2.28 (1.78/2.86)	6.52 ± 3.93	1.43 (1.17/1.62)	2.07 (1.65/2.49)	3.36 ± 1.09*	1.34 (1.17/1.76)
Therapy									
No therapy	98	29.74 (17.85/39.68)	34.31 ± 11.85	2.28 (1.79/2.77)	6.29 ± 4.04	1.39 (1.13/1.58)	2.01 (1.60/2.47)	3.27 ± 1.11	1.34 (1.16/1.68)
Cytostatics[a]	18	27.90 (17.80/39.78)	34.95 ± 14.52	2.13 (1.62/2.66)	6.28 ± 3.05	1.64 (1.40/1.82)	2.03 (1.74/2.36)	3.06 ± 1.02	1.55 (1.20/2.13)
IFN-α[b]	5	49.12 (38.69/66.66)	48.47 ± 19.83	2.52 (1.50/3.10)	7.04 ± 3.18	1.58 (1.34/1.67)	2.30 (1.75/2.81)	3.63 ± 1.17	1.27 (1.13/1.59)
Cytostatics + IFN-α[c]	7	23.55 (14.01/26.82)	22.03 ± 12.46	2.26 (1.99/3.71)	6.47 ± 4.48	1.84 (1.45/2.05)	2.02 (1.78/2.47)	3.38 ± 1.26	1.80 (1.28/2.64)
Therapy > 2 mo[d]	16	35.56 (23.60/40.77)	35.63 ± 11.41	2.09 (1.59/2.74)	5.79 ± 2.65	1.19 (1.02/1.99)	1.87 (1.51/2.23)	2.26 ± 1.02	1.35 (1.15/2.00)
Controls	43	30.41 (21.58/52.75)	48.00 ± 14.69	2.76 (2.11/3.16)	8.21 ± 2.54	1.42 (1.11/1.89)	2.35 (1.96/3.09)	3.91 ± 1.83	1.32 (1.04/1.41)

[a]Untreated patients were compared with patients currently treated with different cytostatics (see "Materials and methods")
[b]Untreated patients were compared with patients currently treated with IFN-α
[c]Untreated patients were compared with patients currently treated with cytostatics in combination with IFN-α (biochemotherapy)
[d]Untreated patients were compared with patients treated more than 2 months ago with any kind of therapy
*$p < 0.05$; **$p < 0.005$; ***$p < 0.0005$; ANOVA analysis (patients vs controls), Mann-Whitney *U*-test (sex, tumor load), ANOVA regression analysis (stage), Bonferroni post hoc test (therapy vs no therapy)

used for statistical comparisons. Normality of the data was tested using the Kolmogorov-Smirnov test, revealing MSFI data of HLA-DR, HLA-DP, and CD11c as normally distributed, whereas MSFIs of HLA-DQ, HLA-I (W6/32), B7.1, B7.2, and CD71 showed skewed data. Hence, regarding these latter parameters all statistical comparisons (Mann-Whitney *U*-test, Bonferroni post hoc test, and ANOVA analysis) were performed with logarithmically transformed data. Multivariate analysis was performed using a general linear model. Differences with a p value less than 0.05 were considered statistically significant. Statistical analysis was performed using SPSS software (SPSS, Chicago, IL).

Results

$CD14^+$ peripheral blood mononuclear cells from 144 patients diagnosed with melanoma as well as 43 healthy controls were analyzed for cell surface expression of HLA class I and class II antigens, CD80/B7-1, CD86/B7-2, CD11c, and CD71.

Decreased expression of HLA class II antigens

Cell surface expression of the class II molecules HLA-DR ($p < 0.0005$), HLA-DQ ($p = 0.006$) and HLA-DP ($p < 0.0005$) was decreased on PBMs from melanoma patients compared with healthy control subjects (Table 1). As shown in Fig. 1A, B, we found a strong association of decreased expression of HLA-DR ($p < 0.0005$) and HLA-DP ($p = 0.008$) with advanced stages of disease. HLA-DQ expression revealed no correlation with disease stages (Table 1). The expression pattern of HLA class II molecules showed no significant changes in regard to age (data not shown), gender, tumor load, or current therapy regimens of the patients tested (Table 1). However, there was a trend to a reduced expression of HLA-DR and HLA-DP in tumor-bearing compared with tumor-free patients (Table 1), that might have reached statistical significance using a higher number of patients with detectable tumor masses. In regard to therapeutic interventions, strongly increased expressions of HLA-DR, HLA-DQ, and HLA-DP could be observed in patients treated with IFN-α in comparison to untreated patients (Table 1). Due to the small number of five patients in this particular subgroup, these changes did not reach statistical significance.

No significant changes in HLA class I expression

In contrast to HLA class II molecules, the expression of HLA class I on PBMs was found without significant differences between melanoma patients and healthy controls (Table 1). Moreover, HLA class I expression showed no correlation to gender or disease stage of the patients. Tumor-free patients revealed a trend to elevated expression levels of HLA class I molecules compared with tumor-bearing patients (Table 1). These differences, however, did not reach statistical significance due to high values for standard deviations and the small number of patients with detectable tumor load. In regard to the mode of therapy we found elevated expression levels of HLA class I in patients treated with IFN-α (Table 1).

Decreased expression of CD86/B7-2 and CD11c

As shown in Table 1, the expression of CD86/B7-2 ($p = 0.001$) and CD11c ($p = 0.13$) was decreased on PBMs from melanoma patients compared with healthy controls. This decrease in expression of both cell surface antigens was associated with advanced disease stages ($p = 0.002$ and $p = 0.031$, respectively; Fig. 2A, B). Additionally, patients with detectable tumor load re-

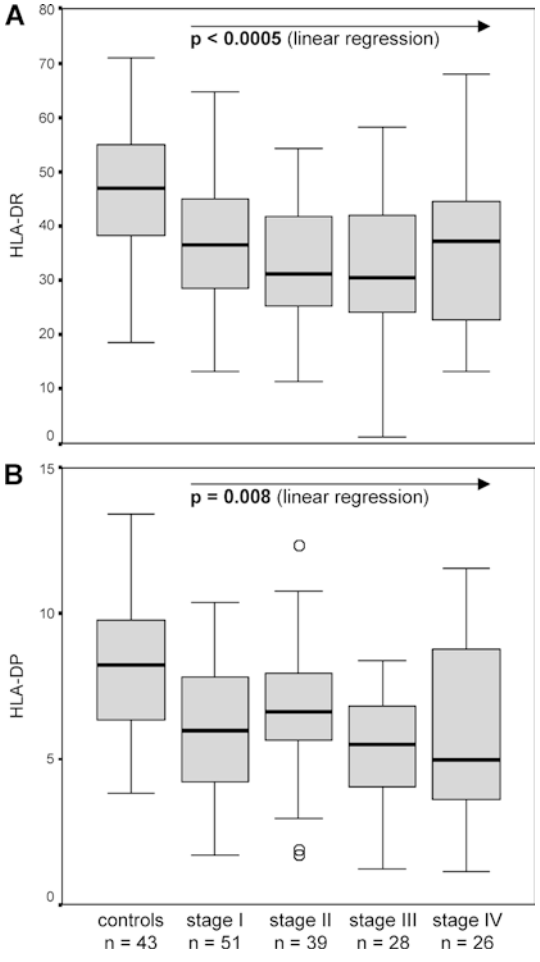


Fig. 1A, B Stage-dependent decrease of surface expression of (**A**) HLA-DR and (**B**) HLA-DP on peripheral blood monocytes from melanoma patients ($n = 144$) and healthy controls ($n = 43$). *Box plots* indicate median *(central bars within boxes)*, upper and lower quartile *(upper and lower bars of boxes)*, and 95% confidence interval *(bars above and below boxes)*. *Stage I/II* primary melanoma, *stage III* regional lymph node and/or in-transit metastases, *stage IV* distant metastases. Os represent outlying cases. Statistical differences between stages were analyzed using ANOVA regression analysis

vealed less CD11c expression than tumor-free patients ($p = 0.022$; Table 1). Neither CD86/B7-2 nor CD11c revealed any significant differences in expression in regard to age (data not shown), gender, or current mode of therapy of the patients (Table 1). However, there was a trend toward up-regulated expression levels of CD86/B7-2 in patients treated with IFN-α in comparison to untreated patients (Table 1). The cell surface expression of CD80/B7-1 showed no significant differences in expression on PBMs from melanoma patients compared with healthy controls (Table 1).

Increased expression of CD71

The cell surface expression of CD71 was elevated on PBMs from melanoma patients compared with healthy control subjects ($p = 0.024$; Table 1). The increase of CD71 expression was associated with advanced stages of disease ($p = 0.006$; Fig. 3). No significant differences in CD71 expression could be detected in regard to age (data not shown), gender, tumor load, and current mode of therapy of the patients (Table 1). Cell surface expression patterns on PBMs from two representative patients with stage I and IV disease, respectively, in comparison to a representative healthy donor are shown in Fig. 4.

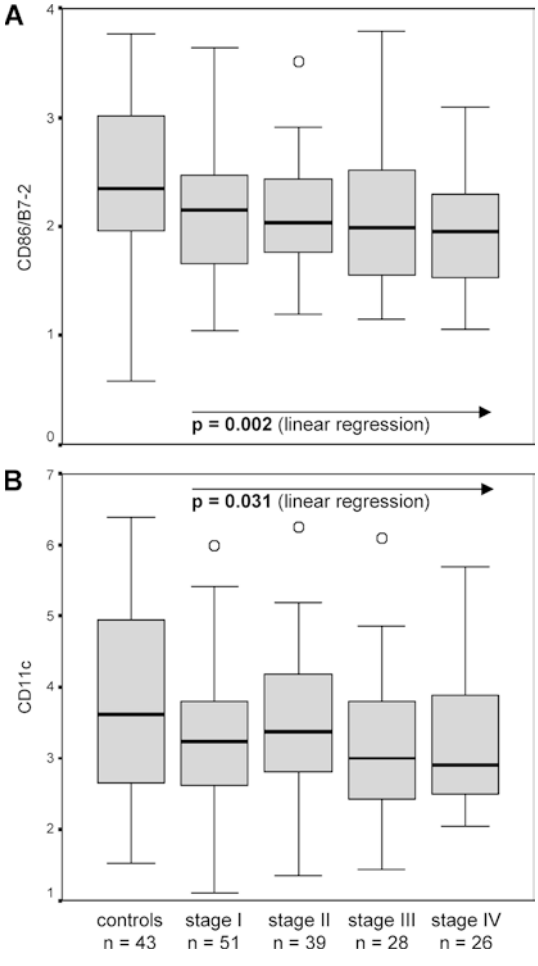


Fig. 2A, B Stage-dependent decrease of surface expression of (**A**) CD86/B7-2 and (**B**) CD11c on peripheral blood monocytes from melanoma patients ($n = 144$) and healthy controls ($n = 43$). *Box plots* indicate median *(central bars within boxes)*, upper and lower quartile *(upper and lower bars of boxes)*, and 95% confidence interval *(bars above and below boxes)*. *Stage I/II* primary melanoma, *stage III* regional lymph node and/or in-transit metastases, *stage IV* distant metastases. Os represent outlying cases. Statistical differences between stages were analyzed using ANOVA regression analysis

Discussion

A defective function of APCs is known to result in an reduced antigen-specific immune response leading to an impaired recognition and eradication of malignant cells. Recently, a decreased antigen-presenting function of dendritic cells has been described in breast cancer patients [10]. Gabrilovich and coworkers found an association between the impaired ability of patients' dendritic cells to stimulate allogeneic T cells associated with reduced cell surface expression of HLA class II and costimulatory B-7 molecules. Though the functional impact of PBMs for the antigen-specific antitumoral immunosurveillance and defense is not yet clarified, defects in the antigen-presenting function of PBMs might possibly impair these mechanisms in cancer patients. Recent studies have described decreased expression levels of HLA-DR on PBMs from patients with malignancies of different origin, such as lung cancer [21], colorectal cancer [21, 22], glioblastoma [33], as well as head and neck cancer [28]. Moreover, the HLA-DR

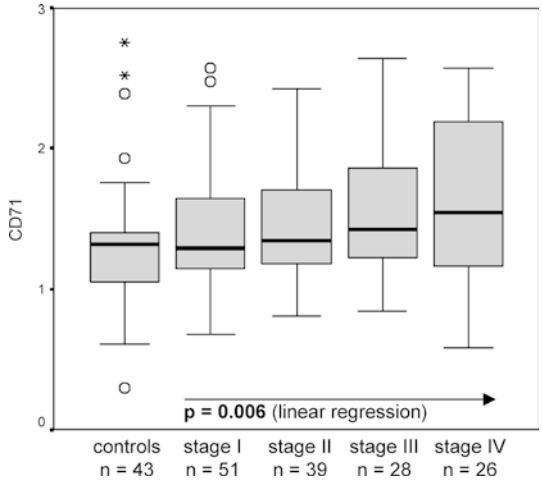
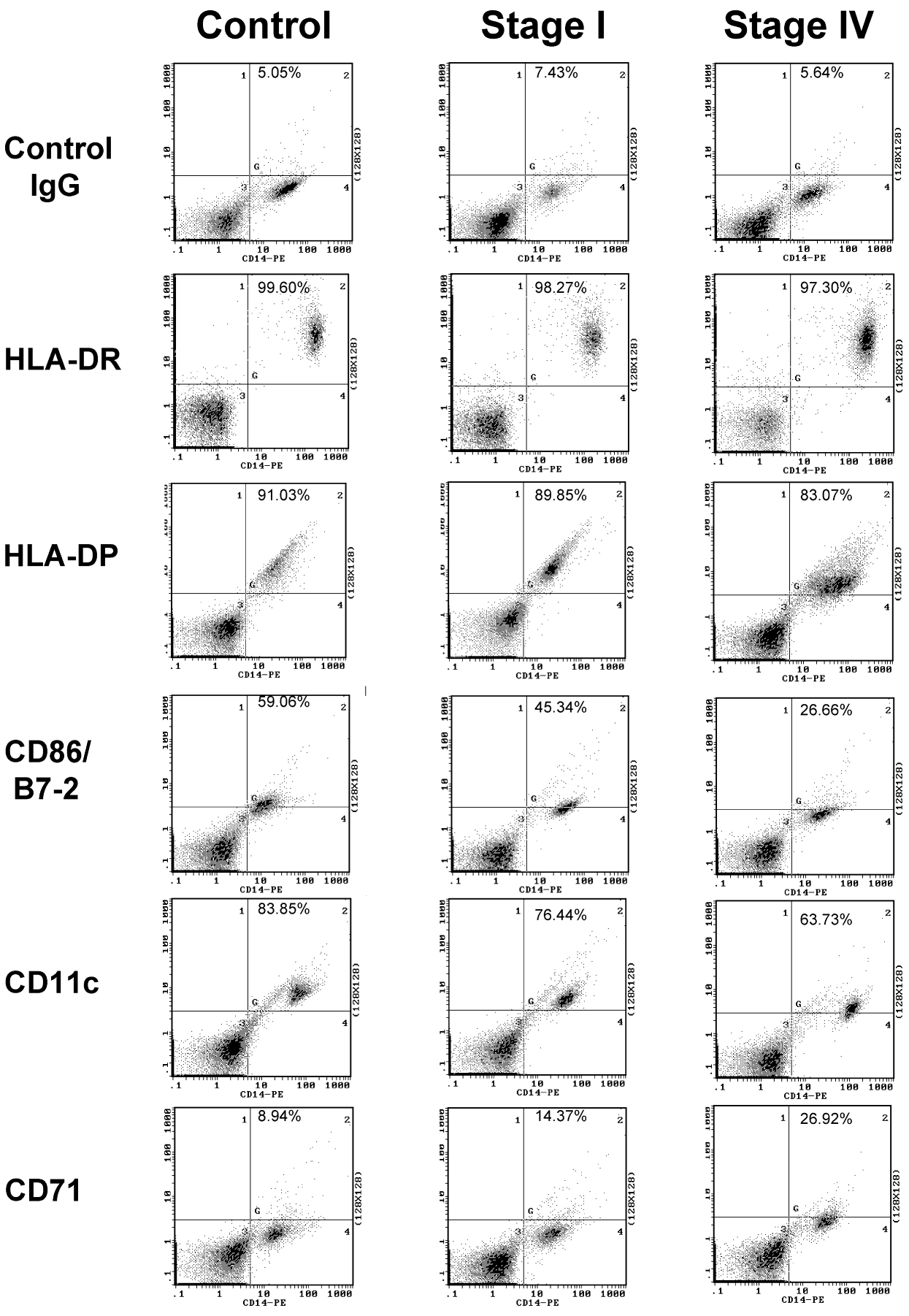


Fig. 3 Stage-dependent increase of surface expression of CD71 on peripheral blood monocytes from melanoma patients ($n = 144$) and healthy controls ($n = 43$). *Box plots* indicate median *(central bars within boxes)*, upper and lower quartile *(upper and lower bars of boxes)*, and 95% confidence interval *(bars above and below boxes)*. *Stage I/II* primary melanoma, *stage III* regional lymph node and/or in-transit metastases, *stage IV* distant metastases. Os and *asterisks* represent outlying cases. Statistical differences between stages were analyzed using ANOVA regression analysis


Control
Stage I
Stage IV
Control IgG
HLA-DR
HLA-DP
CD86/ B7-2
CD11c
CD71
5.05%
7.43%
5.64%
99.60%
98.27%
97.30%
91.03%
89.85%
83.07%
59.06%
45.34%
26.66%
83.85%
76.44%
63.73%
8.94%
14.37%
26.92%
CD14-PE
(12BX12B)

◀

Fig. 4 Cell surface expression patterns of peripheral blood monocytes (PBMs) detected by immunofluorescence double-staining and subsequent flow cytometry analysis. PBMs from two representative melanoma patients with stage I and IV disease, respectively, and a representative healthy donor were processed as described in "Materials and methods." The *x-axis* of each plot indicates the fluorescence intensity of phycoerythrin (PE)-conjugated CD14, the *y-axis* represents the fluorescence intensity of the fluorescein-isothiocyanate (FITC)-labeled antigen indicated on the *left-hand side*. The percentage of specifically stained $CD14^+$ cells is indicated in the *upper right quadrant* of each plot

expression level on PBMs from cancer patients has been shown to be modulated by chemotherapy [21], immunotherapy with IL-4 [25] or IFN-γ [22], as well as by malnutrition [28]. Other authors, however, could not confirm these observations and found PBMs from breast cancer patients in comparison to those from healthy donors not differentially expressing HLA-DR, but showing a higher transmigratory potency when interacting with endothelial cells [11].

A variety of different markers correlating with disease progression has been described for malignant melanoma [4, 14, 26, 27]. Hitherto, the only cancer entity analyzed for a possible correlation between disease progression and HLA-DR expression on PBMs has been cancer of the head and neck, where a correlation could be demonstrated not with tumor stage [28], but with survival of the patients [29]. Therefore, in the present study we analyzed PBMs from a large panel of melanoma patients in different stages of disease and found a strong and stage-dependent decrease in HLA-DR expression in patients compared with healthy controls. This finding corresponds to the strongly reduced serum levels of sHLA-DR we described before in a similar panel of patients [23]. An analogous observation has been made by Ditschkowski and coworkers [6], who reported low sHLA-DR serum values in patients with severe sepsis, corresponding to a reduced HLA-DR cell surface expression on PBMs. These observations suggest that cell surface shedding of HLA-DR from mononuclear cells of the peripheral blood might be a possible source of origin of serum sHLA-DR molecules. However, this potential mechanism has to be further elucidated.

Additionally, the present study demonstrates a significantly reduced expression of other surface molecules belonging to the MHC class II complex, HLA-DQ and HLA-DP, on PBMs from melanoma patients. It has been shown that monocytes can be heterogenously activated by ligation to different MHC class II molecules (HLA-DR, HLA-DQ, and HLA-DP) leading to a differential secretion of monokines which may alter T-cell responses in vivo [20]. Thus, down-regulation of these molecules might contribute to an impaired antigen-presenting function of PBMs in melanoma patients. On the other hand, the low expression pattern of MHC class II might merely reflect an immunosuppressive environment as induced by cytokines such as IL-10 [3, 31] or TGF-β [31].

In regard to costimulatory surface molecules, we found a significantly decreased expression of CD86/B7-2, correlating to advanced stages of disease. In contrast, CD80/B7-1 was only weakly expressed on PBMs, as described previously [19], and revealed no significant differences between melanoma patients and healthy controls. Both molecules of the B7 family, particularly CD86/B7-2, play crucial roles in T-cell activation by APCs [30]. Their importance became evident in the demonstration of T-cell anergy in the absence of B7 signals [15]. Thus, we suggest that the strongly reduced expression pattern of CD86/B7-2 on PBMs from melanoma patients found in our study indicates an impaired costimulation and effector activation of T cells by PBMs, contributing to an impaired antigen-specific immune response status in melanoma patients. Interestingly, it has been shown that low expression of CD86/B7-2 on PBMs was significantly associated with unresponsiveness to vaccination against hepatitis B in chronic hemodialysis patients [12]. This observation underlines the importance of our results supposing a possible impact for therapeutic vaccination strategies in melanoma patients.

To evaluate the status of activation of the PBMs tested, we quantified the cell surface expression of the transferrin receptor CD71 and of the cell adhesion molecule CD11c. Both molecules are known to be up-regulated during PBM activation—e.g., after binding of lipopolysaccharide (LPS), the strongest inducer of an immune response in PBMs by binding to cell surface CD14 [34]. Interestingly, CD71 and HLA-DR expression have been described before to be down-regulated on PBMs from septic shock patients compared with normal subjects, indicating a monocyte anergy [32]. In contrast, our results demonstrate decreased HLA-DR expression combined with a significantly increased CD71 expression on PBMs from melanoma patients compared with healthy controls. The possible impact of this particular PBM expression pattern on the antitumoral immune response needs to be further investigated.

Moreover, we analyzed the impact of therapeutic interventions on the surface expression profile of PBMs. We found no significant differences between patients under chemotherapy compared with untreated patients. Furthermore, no differences could be found between patients without treatment for 2 months and patients without any therapy at all. This observation rules out a long-lasting effect of treatment given to the patients. Due to these findings we conclude that the stage-dependent alterations we detected in the cell surface expression of HLA class II as well as CD86/B7-2 molecules on PBMs is most likely caused by melanoma disease and not by therapeutic interventions. However, other investigators found alterations associated with chemotherapy [21] or immunotherapy [22, 25] in the HLA-DR expression on PBMs from patients suffering from cancer entities different from melanoma. In regard to immunotherapy, which in the panel of patients investigated in the present study is represented by IFN-α

only, we found a trend to up-regulated cell surface expression of HLA class II, CD86/B7-2 as well as CD11c molecules on PBMs from melanoma patients. Due to the small number of patients enrolled in this study while treated with IFN-α, these differences did not reach statistical significance.

In conclusion, this study demonstrates a stage-dependently reduced expression pattern of HLA-DR, HLA-DQ and HLA-DP, CD86/B7-2, and CD11c on circulating PBMs in melanoma patients compared with healthy donors. In contrast, HLA class I and CD80/B7-1 expression revealed no significant differences in patients versus controls. Our results indicate a stage-dependently down-regulated expression pattern of HLA class II and costimulatory molecules on PBMs from melanoma patients that might reflect an ineffective antigen-presenting function of these cells. These mechanisms may contribute to the impaired immune response state known in patients with advanced melanoma.

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