Cytokine production by peripheral lymphocytes in melanoma

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ABSTRACT. *Background*. The differentiation of T cells towards a T helper 1 (Th1) or Th2 phenotype based on their profile of cytokine production, is of great relevance in the regulation of immune responses. We have determined by flow cytometry, the expression of selected Th1 and Th2 cytokines by activated T cells in whole blood samples (WB) from normal donors and from patients with different clinical stages of melanoma in different clinical stages. *Methods*. WB samples from 6 normal donors and 19 patients with melanoma were activated over 4 hours with PMA + ionomycin in presence or absence of a protein secretion inhibitor. Following surface staining (CD3-Cy5+CD8-FITC), fixation and permeabilization, cells were stained with PE-labelled antibodies against Th1 cytokines (IL-2, IFN-γ, TNF-α) and Th2 cytokines (IL-4, IL-10). *Results*. The most relevant results were related to IFN-γ and IL-10 production. The percentage of IFN-γ producer cells was significantly lower in melanoma patients, independent of the stage, than in controls. IL-10 production was significantly increased in melanoma patients with respect to normal donors. *Conclusions*. Our data support the notion that the pattern of cytokines produced by lymphocytes from melanoma patients may help to explain the impairment in their T cell immune response. More extensive studies regarding the pattern of cytokines, not only in peripheral blood, but also in tumour tissue and sentinel lymph nodes, are needed to confirm these data.

Keywords: melanoma, cytokines, Th1, Th2

In 1986, Mosmann *et al.* described two different T helper subgroups, T helper 1 (Th1) and T helper 2 (Th2), based on the pattern of cytokines produced [1]. The same subgroups were later described in humans [2]. Activated Th1 cells secrete IL-2, interferon-γ (IFN-γ) and tumour necrosis factor-α (TNF-α), while activated Th2 secrete IL-4, 5, 6 and 10. Th1 cells provide help for the generation of cytotoxic T cells and generally respond to antigens that lead to delayed hypersensitivity types of immune responses. In contrast, Th2 cells regulate the intensity of immune responses by the secretion of a cytokine, IL10, that inhibits the production of Th1 cytokines. In addition, Th2 T cells provide help to B cells for specific immunoglobulin production, and to respond to antigens that require high antibody levels for foreign antigen elimination, such as in certain parasite infections [3]. Different cytokines can drive the immune response preferentially towards a Th1 or a Th2 response. IL-12, produced by antigen-presenting macrophages is a potent inducer of Th1 responses [4, 5], whereas IL-4 drives differentiation towards a Th2 phenotype type cell [5, 6].

Currently, we know that several dermatological diseases are characterized by the development of either, a Th1- or a Th2-predominant T phenotype. Psoriasis, tuberculoid leprosy and polymorphous light eruption are examples of dermatological conditions with a predominant Th1 response, whereas atopic dermatitis, lepromatous leprosy and systemic lupus erythematosus exhibit a Th2 response [3].

Several studies suggest that the level of certain cytokines may contribute to the identification of melanoma patients at different risks. Porter *et al.* investigated the role of a panel of plasma cytokines in the prognosis of sentinel node-negative melanoma patients [7]. They found that patients with detectable IFN-γ levels were at significantly higher risk of recurrence compared to patients with undetectable levels. Mouawad *et al.* found that serum interleukin-6 concentrations can be considered a predictive marker of recurrent disease in metastatic melanoma patients treated with biochemotherapy [8].

We hypothesized that melanoma in different stages could be associated with different levels of plasma cytokines. The objective of this study was to determine whether there was a correlation between the stage of melanoma and the T cell populations that produce a panel of cytokines (IL-2, IFN-γ, TNF-α, IL-4 and IL-10). The identification of any putative correlation may help us understand the immune mechanisms underlying melanoma progression. Some practical implications may also emerge, such as the identification of new prognostic markers. To this end, the pattern of cytokines produced by plasma T lymphocytes in patients with melanoma in different stages was investigated by means of flow cytometry.

PATIENTS AND METHODS

Patients

Nineteen patients and 6 controls were included in the study. The 6 controls were cleaning personnel who underwent blood tests as part of routine health controls, and who gave informed consent before being included in the present study.

Patients were staged according to the 2001 American Joint Committee on Cancer Staging System for Cutaneous Melanoma (AJCC) [9]. Six patients had stage I melanoma (localized melanoma ≤ 2.0 mm without ulceration, or localized melanoma ≤ 1.0 mm with ulceration), 5 patients had stage II (localized melanoma ≥ 2.01 mm without ulceration or 1.01-4.0 mm with ulceration), and 8 patients had stage IV (disseminated metastatic melanoma).

Methods

Flow Cytometry

Blood from patients and controls was collected in tubes containing heparin, and was processed with a maximum delay of 4 hours.

In order to induce cytokine synthesis, WB was diluted in RPMI 1640 medium (1:1 v/v) and a membrane protein carrier blocker (GolgiStop) was added. Experimental conditions that lead to cell activation were created with the addition of phorbol miristate acetate (PMA) 25 ng/mL and ionomycin 1 μg/mL. All samples were incubated at 37 °C, for 4 hours, with 5-7% CO_2.

Three controls of cellular activation were used in the study:

– A basal activation control was performed diluting WB with RPMI-1640 medium lacking the carrier membrane protein blocker GolgiStop and activation with PMA and ionomycin;

– A control of expression of cellular activation markers on plasma membrane was obtained diluting WB in RPMI lacking GolgiStop, but with the addition of PMA and ionomycin;

– A control of intracellular expression of activation markers was prepared following the same routine described for the cytokines expression study, i.e., diluting WB with RPMI and GolgiStop, and stimulating cells with PMA and ionomycin.The procedure followed in order to determine cytokine synthesis was as follows: 100 μL of activated WB were aliquoted in 12 x 75 mm tubes. Cells were surface marked with 20 μL of CD8-FITC antibody and 20 μL of CD3-Cy5. Cells were fixed and permeabilized with the CytoFix-CytoPerm kit. Intracellular labelling was carried out with 15 μL of specific monoclonal antibody for each of the following cytokines: IL-2, IL-4, IL-10, TNF-α and IFN-γ, as well as the non-specific labelling control, IgG1 and IgG2. All the antibodies for intracellular labelling were conjugated with phycoerythrin (PE).

Control tubes for basal and surface activation were prepared with 100 μL of the corresponding WB dilution and 10 μL of the antibody CD69-Cy5 that detects the surface expression of a cellular activation marker. Samples were lysed and fixed with the TQ-Prep system. Control of the intracellular expression of activation markers was undertaken with 100 μL aliquots of the corresponding WB and 10 μL of CD69-Cy5. Cell permeabilization and fixation was performed with the CytoFix-CytoPerm kit.

All samples were immediately analyzed with a flow cytometer EPICS XL-MCL. Results were quantified with the SYSTEM II analysis program, installed in the flow cytometer computer.

Statistics

GW-BASIC and SPSS-PC statistical programs were used to analyse the experimental data from the study. The following descriptive statistics were calculated: mean (x), standard deviation (s) and number of cases (n). Quantitative variables were compared with the following tests: Fisher´s *F* test for variance analysis and Student´s *t* test.

RESULTS

The results have been analysed following a two-stage procedure. Firstly, we compared the percentage of cells that produce each cytokine, and then we compared the intensity of the cytokine expression by the cells that produced it.

Regarding the percentage of cytokine-producer cells, we have separated the data for each cytokine, and have established the following comparisons:

1. Study group and control group *(table 1)*,
2. Three study subgroups (melanoma stages I, II and IV) and control group *(table 2)*,
3. Localized melanoma (stages I and II) and metastatic disseminated melanoma (stage IV) *(table 2)*,
4. Patients with a disease-free survival (DFS) longer than 2 years and patients with metastatic disseminated melanoma (stage IV).To analyse differences in cytokine expression, the fluorescence intensity of the antibodies specifically linked to cytokine-producing cells was quantified *(table 3)*.

Figure 1 depicts one example of the flow cytometry strategy followed to determine cytokine production by T lymphocytes. Histograms obtained to select the lymphocyte population based on morphological features, and CD4 and CD8 subpopulations based on CD3 and CD8 labelling, are shown. Histograms for quantification of intracellular cytokines are also shown. *Figure 2* depicts the percentages of lymphocytes that produce different cytokines grouped according to melanoma stage.

The most significant results for each cytokine are described in the text. All results and statistical comparisons are covered in detail in the tables.

IFN-γ. There is a significantly higher percentage of IFN-γ-producing CD4 and CD8 T-lymphocytes in controls compared to melanoma patients. Subgroup analysis comparing patients in melanoma stages I, II, IV and controls, demonstrates statistically significant differences (Fisher´s F test) between all groups. Nevertheless, no differences in the percentage of IFN-γ-producing cells were found when the statistics were applied to patients with localized melanoma (stages I and II) and patients with metastatic melanoma in stage IV.

Significant differences in the amount of IFN-γ produced by CD4 lymphocytes were observed among melanoma stages I, II and IV, whereas CD8 lymphocytes were close to, but did not reach the level of significance.

Table 1
Flow cytometry study from peripheral blood activated T lymphocytes. Summary of descriptive statistical data and Fisher test comparing the percentage of T cells that produced each cytokine in control and study groups. Only those cytokines with statistical significant differences between both groups are shown

	Control	Study	Signification
IL-10 (CD4)			
Mean	0.5800	1.9600	F= 9.22
SD	0.2794	1.0871	p= 0.005 **
n	6	19	
IL-10 (CD8)			
Mean	1.6400	0.9942	F= 10.75
SD	0.4906	0.3989	p= 0.003 **
n	6	19	
IFN-γ (CD4)			
Mean	44.9633	6.2458	F= 64.97
SD	20.4983	4.2078	p <0.000 ***
n	6	19	
IFN-γ (CD8)			
Mean	19.4767	7.7763	F=17.7466
SD	6.7720	5.6752	p <0.000 ***
n	6	19	

IL-10. There is a statistically significant difference in the percentage of IL-10-producing CD4 T lymphocytes when comparing controls and melanoma patients. Contrary to what happened with IFN-γ, the percentage of CD4 cells that produced IL-10 was higher in melanoma patients than in controls. However, there happened to be a significantly higher percentage of IL-10-producing CD8 lymphocytes in controls than in melanoma patients. Significant differences were also found in the percentage of IL-10-producing CD4 and CD8 cells comparing controls and melanoma patients in stages I, II, IV. Nevertheless, these differences abate when localized melanoma is compared with disseminated metastatic melanoma. Interestingly, there seems to be a spectrum in the percentage of IL-10producing CD4 cells, so that controls have the lowest percentage, patients with metastatic melanoma have the highest percentage, and patients with localized melanoma have intermediate percentages. This spectrum was not reproduced when we consider the percentage of IL-10-producing CD8 lymphocytes, since controls have the highest percentage, and patients with localized melanoma the lowest.

No differences were observed in the amount of IL-10 produced by CD4 and CD8 lymphocytes.

IL-2. No differences were observed between controls and patients with melanoma. Nevertheless, statistically significant differences were apparent among the control and the three study subgroups. *Table 2* shows a progressive increase in the percentage of IL-2-producing CD4 lymphocytes between control group and stage I melanoma, and between stage I and stage II melanoma. In metastatic melanoma, the percentages are intermediate between stages I and II. No differences were observed between localized melanoma considered as a group, and metastatic melanoma.

The expression of IL-2 by CD8 lymphocytes was on the verge of the statistical significance (p=0.054). No significant differences were found in the rest of the IL-2 comparisons.

No differences were found for IL-4 and TNF-α. Neither did we find any significant differences between patients with localized melanoma and a DFS longer than 2 years, and patients with metastatic melanoma, for any of the cytokines studied.

A larger number of CD8 cells ($p < 0.05$) was observed in metastatic melanoma than in localized melanoma. No differences were observed for the rest of comparisons made with CD8.

In summary, the most interesting observations appeared to be up-regulated IL10 production by CD4 cells and down-modulated expression of IFN-γ in CD4 and CD8 cells of melanoma patients.

DISCUSSION

In the classical paradigm for Th subsets, Th1 and Th2 subsets derive from Th0 cells [3]. Whereas IL-12-induced Th0 differentiation into Th1, IL-4 deviate Th0 into Th2 cells [3, 5, 6]. However, each subtype is characterized by their own pattern of cytokines, IFN-γ is the most characteristic Th1 cytokine, and IL-4 and IL-10 are the most representative Th2 cytokines. To maintain a well-regulated physiological state, there is a balance between both arms: IFN-γ inhibits Th2 cells, and IL-4 and IL-10 inhibit Th1 cells [6, 10, 11]. However, recent studies divide Th cells into 3 subsets. In this new scenario, Th3 or Tr1 cells (regulatory T cells) induce tolerance, and suppress Th1 and Th2 responses *via* IL-10 and transforming growth factor β [12-14]. IL-2 and granulocyte-macrophage colony stimulating factor (GM-CSF) are synthesized by both cell subtypes and enhance the overall immune response [15].

Cytokines are also produced by CD8 cells. Although they are usually characterized by the production of a Th1 cytokine pattern [6], clear evidence has been found of CD8 T cells with a Th2 cytokine pattern, both in mice and humans [16, 17]. Moreover, big differences have been found in cytokine production among different clones that produce either a Th1 or a Th2 cytokine pattern [6]. The interpretations of this complexity ranges from those who consider that there is a model with 2 (Th1/Th2) or more (Th0/Th3) phenotypes, with quantitative differences according to

Table 2
Flow cytometry study from peripheral blood activated T lymphocytes. Summary of descriptive statistical data from control and study subgroups regarding the percentage of T cells that produce each cytokine. Statistic analysis comparing the control group and the 3 study subgroups (stage I, II and IV) among them (Fisher test), and localized melanoma (stages I and II) *versus* metastatic stage IV melanoma (t de Student)

	CONTR	ST I	ST II	STs I + II	Met ST IV	STATISTIC (Cont, I, II, Met) (Fisher)	STATISTIC (I + II) vs Met (Student)
IFN-γ CD4							
Mean	44.9633	4.8167	7.0480	5.8309	6.8163	F= 19.9774	t = 0.49
SD	20.4983	3.5366	4.0453	3.7626	4.9660	p= 0.000	N.S.
n	6	6	5	11	8		
IFN-γ CD8							
Mean	19.4767	5.4717	7.0900	6.2072	9.9338	F= 6.60	t = 1.45
SD	6.7720	3.2535	2.3886	2.8790	7.8608	p= 0.002	N.S.
n	6	6	5	11	8		
IL-10 CD4							t = 0.98
Mean	0.5800	1.8200	1.6700	1.7518	2.2463	F= 3.3783	N.S.
SD	0.2794	1.1817	0.7203	0.9549	1.2547	p= 0.037	
n	6	6	5	11	8		
IL-10 CD8							
Mean	1.6400	0.9800	0.6880	0.8472	1.1900	F= 5.58	t=1.98
SD	0.4906	0.2636	0.2975	0.3056	0.4499	p= 0.005	N.S.
n	6	6	5	11	8		
IL-2 CD4							
Mean	4.4617	5.2030	23.2960	13.4270	10.5175	F= 3.47	t = 0.45
SD	1.7417	3.5255	18.0238	15.0145	12.2481	p= 0.03	N.S.
n	6	6	5	11	8		
IL-2 CD8							
Mean	3.1117	1.3950	4.9960	3.0318	1.8363	F= 4.03	t = 1.06
SD	1.4898	0.8553	3.6987	3.0617	0.9351	p= 0.02	N.S.
n	6	6	5	11	8		
IL-4 CD4							
Mean	1.8100	1.48331	3.2780	2.2990	2.1250	F= 1.04	t = 0.21
SD	0	1.1494	2.5849	2.0522	1.3685	p> 0.05	N.S.
n	1	6	5	11	8	N.S.	
IL-4 CD8							
Mean	1.0400	1.0017	1.1900	1.0872	1.2400	F=0.18	t = 0.53
SD	0	0.3624	0.7422	0.5437	0.7048	p>0.05	N.S.
n	1	6	5	11	8	N.S.	
TNF-α CD4							
Mean	3.6783	5.1450	9.4700	7.1109	7.0875	F = 1.23	t = 0.0008
SD	1.6931	2.2750	8.0539	5.7996	6.4846	N.S.	N.S.
n	6	6	5	11	8		
TNF-α CD8							
Mean	3.8967	3.1100	2.9100	3.0190	3.5963	F = 0.19	t = 0.54
SD	2.7290	2.0494	1.9046	1.8873	2.8037	N.S.	N.S.
n	6	6	5	11	8		
LINFOS CD8							
Mean	25.8933	22.7133	21.1560	22.0054	31.3163	F= 2.61	t = 2.59
SD	4.1090	5.7672	9.1914	7.1473	8.4826	p= 0.077	p < 0.05
n	6	6	5	11	8	N.S.	

lymphocyte development, to those who propose a model without defined subgroups, in which there is a spectrum with different cytokine combinations between both extremes [18].

The majority of the results of the present study can be explained bearing in mind the roles mentioned for IL-10. We found that the CD4 population that produced IL-10 was significantly larger in melanoma patients than in controls. This explains why the melanoma patients had a lower percentage of CD4 and CD8 lymphocytes that produced IFN-γ. An alternative explanation for the decrease in IFN-γ-producing cells in the blood of melanoma patients compared to control individuals should be considered. It may not be related to the newly IL-10-producing cells, but simply due to the mobilization of IFN-γ in lymph nodes and tumoral tissue. Thus, this down-regulation would be non-specific to melanoma tumours.

The percentages of T lymphocytes that produce IFN-γ are similar in patients with melanoma in different stages. Nevertheless, although there are no statistically significant differences, there is a gradient in IL-10-producing CD4 lymphocytes according to the stage of melanoma, so that

Table 3
Flow cytometry study from peripheral blood activated T lymphocytes. Summary of descriptive statistical data from control and study subgroups regarding cytokine expression intensity. Statistic analysis comparing the control group and the 3 study subgroups (stage I, II and IV) among them (Fisher test), and localized melanoma (stages I and II) versus metastatic stage IV melanoma (t de Student)

	ST I	ST II	STs I + II	Met ST IV	STATISTIC (Cont, I, II, Met) (Fisher)	STATISTIC (I + II) vs Met (Student)
IFN-γ CD4						
Mean	4.2300	11.3400	7.4618	9.1950	F= 4.405	F=0.572
SD	2.8677	5.2711	5.3863	4.1989	p= 0.030	p=0.460
n	6	5	11	8		
IFN-γ CD8						
Mean	4.2917	12.2320	7.9009	8.7937	F= 3.548	F=0.111
SD	3.3372	5.9399	6.0726	5.3349	p= 0.053	p=0.744
n	6	5	11	8		
IL-10 CD4						
Mean	2.9667	3.7800	3.3364	4.3462	F= 0.711	F=1.067
SD	2.1056	1.1776	1.7181	2.5568	p= 0.506	p=0.316
n	6	5	11	8		
IL-10 CD8						
Mean	3.1700	3.8580	3.4827	4.2950	F= 0.439	F=0.646
SD	1.0316	1.1749	1.1012	3.1240	p= 0.652	p=0.433
n	6	5	11	8		
IL-2 CD4						
Mean	3.2933	5.2020	4.1609	3.6000	F= 1.921	F=0.432
SD	0.7959	2.0977	1.7522	1.9518	p= 0.179	p=0.520
n	6	5	11	8		
IL-2 CD8						
Mean	3.1550	5.5520	4.2445	3.4063	F= 3.526	F=0.944
SD	0.2951	3.0360	2.3016	0.8974	p= 0.054	p=0.345
n	6	5	11	8		
IL-4 CD4						
Mean	2.1700	2.4040	2.2764	2.1063	F= 0.978	F=0.924
SD	0.4974	0.2942	0.4162	0.3239	p=0.397	p=0.350
n	6	5	11	8		
IL-4 CD8						
Mean	2.6650	3.0560	2.8427	2.5150	F=1.437	F=1.535
SD	0.6986	0.6601	0.6782	0.3602	p=0.267	p=0.232
n	6	5	11	8		
TNF-α CD4						
Mean	4.7017	5.0480	4.8591	4.2588	F=1.311	F=2.268
SD	0.8880	0.6299	0.7653	0.9752	p=0.297	p=0.150
n	6	5	11	8		
TNF-α CD8						
Mean	4.4983	4.3240	4.4191	3.6450	F=1.325	F=2.721
SD	1.0406	0.4885	0.8033	1.2473	p=0.293	p=0.117
n	6	5	11	8		

patients with melanoma in a more advanced stage have a higher number of cells that produce IL-10. In the field of cancer, a relationship has been found between increased expression of IL-10 and several tumours, including melanoma and melanoma metastases [19-21]. Two mechanisms have been suggested; either IL-10 may act as a growth factor for neoplastic cells [22], or IL-10 produced in the vicinity of the tumour or by the tumour itself, may hamper the induction or the effector arm of the antitumour immune response [23, 24].

IL-10 can suppress T cell responses to melanoma [25], based on its action on different crucial points of the immune response. IL-10 has been shown to downregulate expression of MHC classes I and II and ICAM-1 [26]. Other investigators have demonstrated that incubation of melanoma cells with IL-10 led to a 100% inhibition of autologous cytotoxic T-cell lymphocyte-mediated tumor-specific lysis and a 50% reduction in MHC class I expression [27]. In addition, IL-10 inhibits the presentation of tumoral antigens by the epidermal antigen-presenting cells [28, 29]. It has been shown that IL-10-treated human DC are able to induce melanoma antigen-specific anergy in both primed and naive (CD45RA+) CD8 cells [30]. It is noteworthy that T cell activation in the presence of IL-10 may induce a non-response or anergy state that cannot be reversed if IL-2 is present or with anti-CD3 or anti-CD28 stimulation [31]. IL-10-mediated anergy may be associated with the induction of a regulatory T cell population, which produces high levels of IL-10 and TGF-β, and which can suppress *in vivo* and *in vitro* antigen-specific responses [32-35].

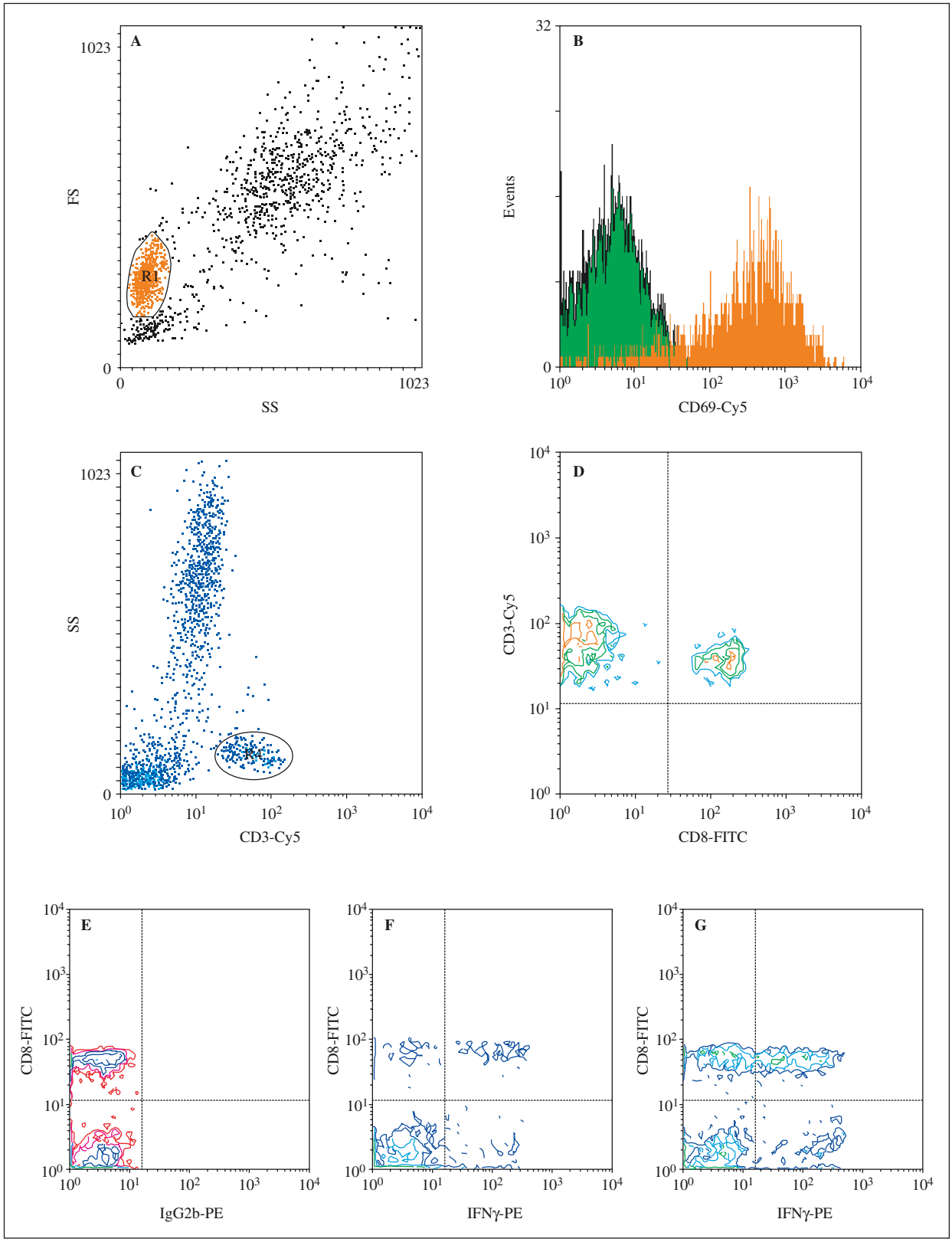

Figure 1

Illustrative example of the flow cytometry strategy followed to determine intracellular cytokines in peripheral whole blood lymphocytes. **A)** Biparametric histogram of size (FS) versus complexity (SS) to isolate the lymphocyte population. **B)** Cellular activation control: superposition of distribution histograms in lymphocytes activated with PMA and ionomicine (red line) and and non-activated controls (green line). **C)** Complexity histogram (SS) against the red fluorescence of the CD3-Cy5, to select the population of T lymphocytes (CD3+) for cytometric analysis. **D)** Biparametric histogram of red fluorescence (CD3-Cy5) against green fluorescence (CD8-FITC) to identify the CD4 and CD8 T lymphocytes subpopulations: CD4 lymphocytes correspond to the CD3+CD8- population (left upper quadrant) **E)** Biparametric histogram of green fluorescence (CD8-FITC) against orange fluorescence (IgG2b irrelevant antibody), showing the cursor adjustment to determine the negative population (left quadrants) in the analysis of T lymphocytes. **F)** and **G)** Biparametric histograms of green fluorescence (CD8-FITC) against orange fluorescence (IFN-γ-PE), to simultaneously show the expression of IFN-γ in CD4 and CD8 T lymphocytes. Examples of melanoma patients in stage I and IV respectively.

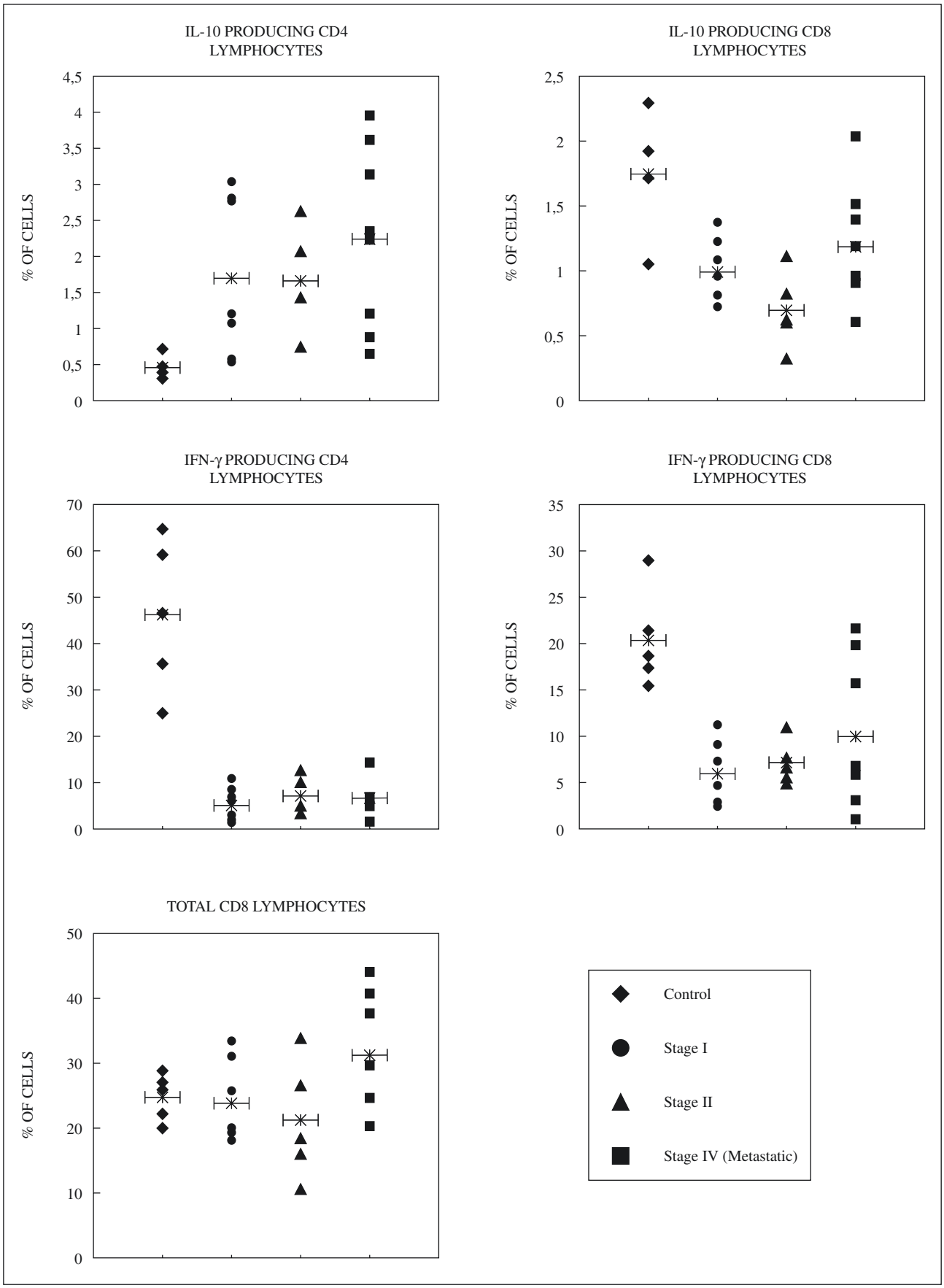

Figure 2

Intracellular cytokine expression in peripheral whole blood CD4 and CD8 T lymphocytes from healthy controls and melanoma patients. Graphs show the percentages of positive cells for each cytokine and in each patient (vertical axis) depending on the stage of disease: I, II or IV melanoma stages or control group (horizontal axis). Each dot represents a patient. Horizontal bar shows the mean.

Though it may seem contradictory, it has been reported that IL-10 has stimulatory properties on CD8 cells: attracting, activating and favoring their proliferation [36-39]. Moreover, evidence from tumours controlled by NK cells, suggests that IL-10 may act to inhibit tumour growth or metastasis *via* an enhanced NK cell antitumour response [40].

Although this is a preliminary study with a reduced number of patients, some interpretations can arise from the group of patients with melanoma. An interesting finding was the increase in the Th1 immune response in stage II compared to stage I (*table 2* shows a large increase in the percentage of IL-2-producing cells and low increase in the percentage of TNFα- and IFNγ-producing cells, and *table 3* shows an increase in the amount of IFNγ produced by T lymphocytes). One might speculate that IL-10producing CD4+ cells, which are increased in the blood of patients with metastatic melanomas, down-regulate the Th1 response observed in stage II patients.

New avenues of research in this field are possible. Firstly, important differences in the immune response between patients may complicate the interpretation of data. These differences can even be observed in the cytokine levels of controls. This is due to the many different situations (infections, autoimmune diseases, etc.) that may modify the immune response independent of the presence of a tumour or not. Secondly, it would be interesting to carry out longitudinal studies, performing several determinations at different time points in the same patient. This would allow a better analysis of the Th1/Th2 pattern in the course of disease. Lastly, it is important to study the modifications in the pattern of cytokines produced by patients treated with immunotherapy, chemotherapy or any combination regimen. Changes in the pattern of cytokines produced by T lymphocytes may be important for the prognosis of patients, or to modify the treatment they are receiving.

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