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Original Paper

GM-CSF Can Cause T Cell Activation; Results of Sequential Chemo-immunotherapy

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HUMAN GRANULOCYTE macrophage-colony stimulating factor (GM-CSF) is well known to induce proliferation and differentiation of myeloid progenitor cells and has profound effects on the effector function of granulocytes and macrophages, comprising cytotoxicity and antigen presenting capacity, the latter also of dendritic cells (DC) [1, 2]. Several *in vitro* and animal *in vivo* data indicate that T cells may be activated as well, either directly via the GM-CSF receptor on T cells, or indirectly via antigen presenting cells [2, 3]. In addition GM-CSF with low-dose IL-2 induce T lymphocyte lymphokine activated killer (T-LAK) cells without induction of natural killer-LAK (NK-LAK) with their inherent toxicity as vascular leak syndrome [4–6]. We report here T cell activation in humans as a result of chemo-immunotherapy consisting of sequential DTIC, GM-CSF, IL-2 and IFNα. The idea was that DTIC would induce tumour cell antigen release, GM-CSF antigen presentation, low-dose IL-2 further proliferation and differentiation of activated T cells without activation of NK cells and IFNα immunosensitivity of tumour cells. After DTIC and 6 days GM-CSF considerable T cell activation was found.

PATIENTS AND METHODS

Patients and treatment

All patients included in the study had a histologically confirmed diagnosis of malignant melanoma, originating in the skin or eye. They had progressive metastatic disease, adequate bone marrow, liver and renal function, performance score WHO 0–2, and signed informed consent. Patients were excluded in case of uncontrolled other cancers, previous systemic treatment for metastatic melanoma, use of immunosuppressive medication, symptomatic brain metastasis or anti-HIV antibodies. The study protocol was approved by the hospital's medical ethical committee. Treatment consisted of dacarbazine (DTIC) 800 mg/m^2 intravenously (i.v.) day 1, GM-CSF (Molgramostin, Schering Plough, Amstelveen, The Netherlands) 2.5 μg/kg subcutaneously (s.c.) day 2–12 (10×), low-dose IL-2 (Aldesleukin, Chiron Europe, Amsterdam, The Netherlands) 1.8 MIU flat s.c. day 8–18 (10×) and IFNα (interferon alpha-2b, Schering Plough) 6 MU flat s.c. day 15–19 (5×). After two cycles patients were evaluated. If they had no progressive disease (PD) or dose limiting toxicity at least another two cycles were given.

Supportive care consisted of prophylactic use of ondansetron for DTIC-induced emesis, metoclopramide for other periods of nausea and vomiting, and acetaminophen for flu-like symptoms of the immunotherapy part.

Immunomonitoring

T cells (CD3+), its subsets (CD4, CD8) were studied for activation (double staining with DR), as well as the number of NK cells (CD16+CD56 positive) by immunophenotyping (FACS) on days 1, 8, 15, 20 and 22 of treatment. sIL-2R and sCD8 were studied in plasma samples by Elisa.

Statistical analysis

Differences between absolute numbers of circulating cells and cytokines were evaluated by Student's *t*-test. P values <0.05 were considered significant.

RESULTS

Patients

Patient characteristics are shown in Table 1. 10 of 37 patients with a median of 2.5 metastatic sites showed responses (27%); 4 CR, 6 PR. With a median follow up of 18 months, median survival was 7 months and 1 year survival was 20%. Limited toxicity (≤grade 2 fever, malaise, fatigue, local reactions) was found and the whole treatment could be given on an outpatient basis.

Immunomonitoring

Results of immunophenotyping are shown in Table 2: a significant increase in CD3/DR, CD4/DR and CD8/DR T cells was found at day 8 after DTIC with GM-CSF, with a further increase of CD3/DR and CD4/DR at day 15 after administration of IL-2. Before the second cycle values returned to start levels. An increase in NK cells was found on days 15 and 20 after IL-2 treatment. sIL-2R (Table 3) also showed a significant increase at day 8, further increasing during GM-CSF+IL-2 treatment. No increases were seen after chemotherapy and/or G-CSF in 6 other tumour

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Table 1. *Patient characteristics*

Characteristic	No.
Male/female	16/21
Age (median, years) range	49.3 (20–67)
Performance status	
WHO	25
WHO 1,2	12
Primary tumour organ	
Skin	32
Eye	5
No. of disease sites	
1	10
2	9
≥3	18
Mean	2.5

Table 2. *Results of immunophenotyping*

	CD3/DR	CD4/DR	CD8/DR	NK
Before	34	17	14	173
D8, GM-CSF	131*	57†	44†	196
D15, GM+IL-2	223†	166†	46†	265*
D19, IFNα	87*	69†	33	235*
D22, start cycle 2	52	28*	21	222

Results are given as mean absolute numbers/μl blood; *Significantly higher than before treatment ($P<0.05$). †$P<0.01$. GM-CSF, granulocyte macrophage-colony stimulating factor; IL-2, interleukin-2; IFNα, interferon alpha; NK, natural killer.

patients. sCD8 did not show a significant increase (results not shown).

DISCUSSION

Sequential chemo-immunotherapy consisting of sequential DTIC i.v. and GM-CSF, low-dose IL-2 and IFNα subcutaneously could be given to patients with metastatic melanoma in an outpatient setting with limited toxicity (≤grade 2 fever, malaise, fatigue, local reactions). It was clearly active with 27% responses including CR in 4/37 patients with an interesting subgroup of patients with survival longer than 1 year.

Immunomonitoring showed significant T cell activation after DTIC followed by 6 days GM-CSF s.c.: significant rises in CD3/DR, CD4/DR, CD8/DR T cells and sIL-2R. An increase in sIL-2R was not found after chemotherapy followed by G-CSF in other tumour patients, as has been previously reported [3, 4]. We assume that this T cell activation was not a direct effect of GM-CSF on T cells, as human T cells lack GM-CSF receptors [7]. Indeed, an indirect effect via antigen presenting cells (DCs and macrophages), which are produced from myeloid precursor cells and are activated by GM-CSF, seems more likely, as suggested by experiments in GM-CSF knock-out mice [8]. However, not only tumour cell antigens, but also auto-antigens will be efficiently presented to T cells, which has the risk of activation of autoimmune disease [9].

Table 3. *sIL-2R during first cycle*

	Melanoma	Other tumours
	DTIC+GM-CSF	CT+G-CSF
Before	800±1000	980±1000
Day 8	3000±1600†	1090±1400
Day 15*	3600±1600†	
Day 19	2400±1400	
Day 22	2000±1200	

*GM-CSF 2.5 μg/kg was given on days 2–12; IL-2 1.8 MIU days 8–18; IFNα 6 MIU days 15–19. †Significant increase ($P<0.01$) compared to values before. DTIC, dacarbazine.

Addition of low-dose IL-2 (1.8 MIU) s.c. induced a further increase in CD3/DR, CD4/DR and sIL-2R, without a further increase in CD8/DR. In a current trial we are exploring the effect of an increase in GM-CSF (up to 5 μg/kg) and IL-2 (up to 4 MIU/m^2) to investigate whether CD8 + T and/or NK cell activation can be induced as well. No biopsies were taken form the metastases, so an effect of tumour cell infiltrate by GM-CSF and IL-2 or IFNα on expression of HLA class I and II or adhesion molecules was not documented. It will be important in future studies to demonstrate T cell reactivity (both CD4 + and CD8 +) to melanoma associated antigens in responding patients.

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