Effects of cadmium and Gc-Macrophage Activating Factor (GcMAF) on intracellular HIV targets in normal and transformed human breast cells

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BOX 1

CASE REPORT n. 1.
GcMAF administration in an AIDS patient with Bb/FF vitamin D receptor haplotype is associated with increased blood monocyte and CD4+ cell count, decreased viremia, and significant improvement of health conditions.

It was demonstrated that administration of GcMAF to asymptomatic HIV-1-infected patients elicited therapeutic results (J Med Virol 2009; 81:16-26). At the XVIII Intl AIDS Conf, it was demonstrated that the response to GcMAF was associated with vitamin D receptor (VDR) polymorphisms (abstr THPE0051). Here we describe the case of an AIDS patient who self-administered GcMAF. The patient, now 44 years old, had been diagnosed HIV-positive in 1984, and full-blown AIDS in 2001. Since 2003 the patient reported peripheral neuropathic pain and invalidating limb stiffness. In 2009-2010 the patient developed resistance to conventional antiretroviral (ARV) therapy and observed a decline of general health conditions with persistent fatigue accompanied by very low CD4+ cell count (40 cells/ml) and viremia values of about 160,000 copies/ml. Determined VDR haplotype was Bb/FF. In 2010, the patient autonomously purchased GcMAF from www.gcmaf.eu and, after one month of abstinence from ARV therapy, in May 2010 began injecting 100 ng GcMAF/week intramuscular for 10 weeks. Blood monocyte count (expressed as %) increased, and reached a peak of 14% after 4 injections; it returned to normal values after ceasing GcMAF administration. CD4+ cell count increased up to 298 cells/ml after 10 injections. Viremia decreased to 2,343 copies/ml. The patient, interviewed in December 2010, reported significant and stable improvement of general conditions; in particular, the absence of the peripheral neuropathic pain and limb stiffness that had severely decreased quality of life. The patient reported conducting a very active life-style and enjoying excellent health conditions. These observations might prove encouraging in testing the effectiveness of GcMAF also in AIDS patients.

CASE REPORT n. 2.
Observation of symptoms compatible with immune reconstitution inflammatory syndrome in an AIDS patient with BB/FF VDR haplotype, treated with GcMAF.

The patient, now 39 years old, had been diagnosed HIV-positive in 1993, and full-blown AIDS in 2002. She was on HAART since 2002, experiencing common side effects such as, diarrhoea, bone pain, signs of hepatotoxicity and depression. Determined VDR haplotype was BB/FF. In January 2011, she began weekly i.v. treatment with 100 ng GcMAF (www.gcmaf.eu) under medical supervision. After the third injection of GcMAF, although she had previously discontinued HAART, the patient reported flushes immediately after each GcMAF injection, muscle pain all over her body followed by the onset of high fever (40°C) accompanied by diffuse burning and stitching pain, extreme skin sensitivity to touch, severe headaches and eye pain perceived as "pressing". Symptoms were relieved by administration of dexibuprofen, 300 mg every 12 hours. Ever since the patient reports constant improving of her general conditions. (Case report courtesy of Dr. Santos-Koenig, Vienna, Austria). These side effects, although unwelcomed, appear to demonstrate that GcMAF actually induces immune system reconstitution.

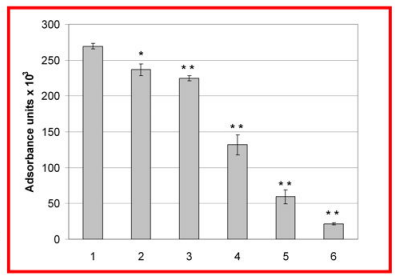
Introduction

Breast cancer is of particular importance among non-AIDS defining cancers. In fact, even though breast cancer risk is significantly lower for women with HIV infection compared to the general population (Cell Death and Disease 2010 1, e30; doi:10.1038/cddis.2010.8. PLoS One. 2010 Dec 16;5(12):e14349), patients with HIV infection present with more advanced stage and aggressive disease, and they also have poor chemotherapy tolerance. This led us to the search for an alternative therapeutic approach targeting both immunodeficiency and cancer. We had previously studied the effects of a known human carcinogen, cadmium (Cd), on cell proliferation, angiogenesis, and on two of the main intracellular targets of HIV, heat shock protein 90 (hsp90, targeted by Tat protein, known to be imported into the nucleus of human breast cancer cells), and poly(ADP-ribose) polymerase (PARP, involved in DNA repair and oxidative stress associated with HIV infection). Here we report the latest results obtained with GcMAF, a protein proven effective in the treatment of breast cancer and HIV infection. Thus, it was demonstrated that GcMAF eradicates HIV infection in asymptomatic HIV-1-infected patients (J Med Virol 2009; 81:16-26), and here we report for the first time its effects in full-blown AIDS patients (BOX 1). GcMAF was also reported to eradicate advanced breast cancer (Int J Cancer 2008; 122:461-7). Here we demonstrate that the anti-cancer effects of GcMAF can be attributed to multiple actions: direct inhibition of cancer cell proliferation, reversal of cancer cell malignant phenotype, and stimulation of macrophage differentiation. GcMAF used in our experiments was kindly donated by www.gcmaf.eu.

Inhibition of human breast cancer cell proliferation

Human breast cancer cell (MCF-7) proliferation was significantly inhibited by 72 h incubation with GcMAF in a dose-dependent manner. Fig. 1, shows that GcMAF (Column 2, 0.4 ng/ml. Column 4, 40 ng/ml) achieved inhibition similar to that of vitamin D (1,25(OH)2D3. Column 3, 100 nm. Column 5, 1μM), a known inhibitor of MCF-7 cell proliferation (BMC Genomics. 2009 Oct 28;10:499). Vitamin D (1 μM) and GcMAF (40 ng/ml) administered together led to almost total inhibition of cell proliferation (Column 6. * indicates $p < 0.02$ *vs* control, *i.e.* column 1; * *indicates $p < 0.01$ *vs* control, *i.e.* column 1). The lowest inhibiting concentration of GcMAF (0.4 ng/ml) was much lower than that reported by Gregory et al. in LnCaP prostate cancer cells (PLoS One. 2010 Oct 18;5(10):e13428). This difference might be ascribed to different sensitivities of cultured cells to GcMAF (see Fig. 3).

FIG. 1



Reversal of human breast cancer cell malignant phenotype

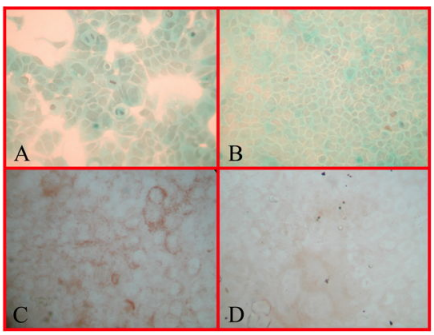


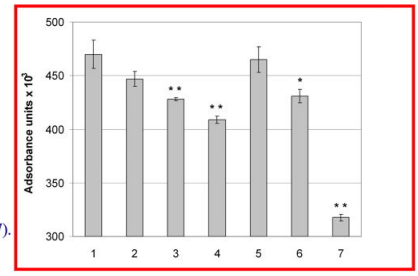
FIG. 2

Fig. 2, shows Papanicolaou staining of MCF-7 cells (magnification X 40) before (A), and after (B) 72 h exposure to GcMAF. In panel A, large nuclei and several mitoses are clearly visible. Untreated MCF-7 cells appeared inhomogeneous in morphology and size with polymorphic large and small cells represented. Cells grew one on top of the other forming typical tumour clusters and the large empty spaces between clusters indicate poor adherence to the well surface. In panel B (MCF-7 cells treated with 40 ng/ml GcMAF), cells grew in monolayer and no clusters could be observed. Cells were much smaller, regularly polygonal and uniform in morphology and size. Cells appeared well adherent to each other and to the well surface. This phenomenon can be interpreted as if GcMAF induced reversal of cancer cell malignant phenotype, a phenomenon confirmed by the study of vimentin expression. Immunohistochemical analysis demonstrated that 72 h exposure to GcMAF significantly decreases vimentin expression in MCF-7 cells (panel D) as compared to control (panel C). These data, confirmed by western blot analysis, are consistent with GcMAF-induced reversal of epithelial-mesenchymal transition, a hallmark of breast cancer malignant progression.

Effects of GcMAF on normal breast cells and monocytes/macrophages

GcMAF did not modify normal human breast cell (MCF-10) proliferation whereas it inhibited proliferation of the human monocytes/macrophages cell line Mono Mac 6 (MM6). Inhibition of MM6 cell proliferation is consistent with the known effects of GcMAF on macrophage activation. In fact, it was demonstrated that monocytes/macrophages activated by GcMAF immediately blocked DNA synthesis and rapidly differentiated (J Med Virol 2009; 81:16-26). Fig. 3, shows dose-dependent inhibition of MM6 proliferation (Columns 2 - 4, GcMAF 0.4, 4, 40 ng/ml. *indicates $p < 0.05$ *vs* control, *i.e.* column 1; * *indicates $p < 0.01$ *vs* control, *i.e.* column 1). The effects of GcMAF on MM6 cells were less pronounced than those observed in MCF-7 cells as if different cells lines showed different sensitivities to GcMAF. It is worth nothing that MM6 VDR haplotype is Bb/FF (see BOX 1).

FIG. 3



Mono Mac 6 cells as a tool to test GcMAF-like activity

We recently proposed chick embryo chorionallantoic membrane (CAM) assay as a simple method to determine the relative potencies of different GcMAF preparations and their stability (Cancer Immunol Immunother 2010 doi 10.1007/s00262-010-0953-7). Here we demonstrate that also the MM6 cell line is an excellent system to test GcMAF activity *in vitro*. Thus, we tested a raw preparation obtained in our laboratory, putatively containing GcMAF, on MM6 cell proliferation. This preparation was obtained treating colostrum with kefir grains. It is known that kefir modulates the immune response in mice, increasing the phagocytic activity (*i.e.* activating) of peritoneal and pulmonary macrophages (Immunobiology 2006; 211:149-56). It is conceivable that microorganisms of kefir grains could convert milk Gc protein into active GcMAF due to their enzymatic activities. We used colostrum instead of milk because colostrum is richer in Gc protein (J Nutr Biochem 1992; 3:498-502). Freshly collected colostrum was incubated for 24 h with kefir grains. The liquid supernatant was collected, filtered and incubated with MM6 cells for 72 h as in the experiments reported in Figs 1 and 2. Increasing concentration of kefir supernatant (expressed as total protein content, *i.e.* 4.8 – 480 μg/ml. Fig. 3, columns 5 - 7) significantly inhibited MM6 proliferation in a manner similar to that observed with GcMAF.

GcMAF and cadmium in MCF-7-induced angiogenesis

We had previously demonstrated that exposure of MCF-7 cells to subtoxic levels of Cd inhibited their angiogenic potential, suggesting the possibility that Cd might exert a paradoxical effect in breast cancer: on the one hand, it could promote carcinogenesis, and, on the other hand, it could delay the onset of tumours by inhibiting breast cancer cell-induced angiogenesis (J Environ Pathol Toxicol Oncol 2009; 28:85-8). Since Cd and GcMAF exerted similar effects on MCF-7 and on MCF-7-induced angiogenesis, we hypothesize that hsp90 and PARP are involved also in the GcMAF signalling pathway.

Conclusions

Our data demonstrate that GcMAF exerts multiple effects on normal and transformed cells; these effects are consistent with, and could be responsible for, its well documented anti-cancer effects. Our observations (BOX 1) also suggests that GcMAF might prove useful in AIDS patients. In addition, the data presented in J Med Virol (2009; 81:16-26) and in BOX 1, provide experimental evidence for the words "Our immune system will get rid of the virus within a few weeks, if you have a good immune system", thus reversing the long-assumed cause-effect relationship between HIV and AIDS.

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