Intracerebral HIV glycoprotein (gp120) enhances tumor metastasis via centrally released interleukin-1

Deborah M. Hodgson a,*, Raz Yirmiya d, Francesco Chiappelli a,b, Anna N. Taylor a,c

a Dept. of Neurobiology and Brain Research Institute, School of Medicine, University of California, Los Angeles, CA 90095, USA
b Dept. of Neurobiology and Brain Research Institute, School of Dentistry, University of California, Los Angeles, CA 90095, USA
c West L.A. DVA Medical Center, Los Angeles, CA 90095, USA
d Dept. of Psychology, Hebrew University of Jerusalem, Mt. Scopus, Jerusalem 91905, Israel

Accepted 7 October 1997

Abstract

Infection with the human immunodeficiency virus (HIV) is associated with a high incidence of cancers. This relationship does not appear to be due to a direct effect of the virus, and may be mediated by neuroimmune interactions since the HIV glycoprotein, gp120, enters the brain soon after infection with HIV, and intracerebroventricular (i.c.v.) infusion of gp120 suppresses aspects of cellular and tumor immunity. It has been speculated that this suppression may be attributed to the release of interleukin-1 (IL-1) in the brain induced by gp120. Using in vivo tumor model, we examined the effect of centrally administered gp120 on tumor metastasis and lung clearance of mammary adenocarcinoma (MADB106) tumor cells in rats, and the role played by brain IL-1 in mediating these effects. We demonstrate that central administration of gp120 (4 µg) significantly (p < 0.05) increased the retention of tumor cells in the lungs and significantly (p < 0.02) enhanced the development of tumor metastases. Central administration of IL-1β (10 ng) also significantly (p < 0.05) increased retention of tumor cells in the lungs. The effect of gp120 on lung retention of tumor cells was blocked by co-administration of α-melanocyte stimulating hormone (α-MSH, 20 ng), a hormone that blocks many of the biological effects of IL-1, or the IL-1 receptor antagonist (50 µg). Given that systemic administration of gp120 or IL-1β had no effect on the retention of tumor cells in the lungs, these findings indicate that gp120-induced secretion of IL-1 within the brain most likely mediates the effects of gp120 on tumor metastasis. These findings suggest a possible neuroimmune mechanism to account for the increased incidence and aggressiveness of tumors in HIV-infected patients. © 1998 Elsevier Science B.V.

Keywords: gp120; HIV; MADB106; IL-1; Tumor; Metastasis

1. Introduction

Human-immunodeficiency-virus (HIV) infection is associated with an increased incidence of malignant neoplasms. In addition to Kaposi’s sarcoma and non-Hodgkin’s lymphoma, which are particularly prevalent in AIDS patients, oral, rectal, testicular, and lung cancers have all been found to be associated with HIV infection [1,10,19,20,50]. In each instance the cancer is particularly aggressive and resistant to treatment [11,15]. The relationship between HIV infection and tumorigenesis appears to be indirect since HIV, in contrast to human oncoretro-viruses, does not have in-vitro-transforming capacity nor is the HIV provirus found in any of the tumors associated with AIDS [15,19,50]. Tumor immunity is, however, compromised in HIV-infected individuals. Progression from HIV infection to AIDS is associated with a dramatic decline in the number and activity of natural killer (NK) cells, which are critically involved in the surveillance and early eradication of tumor cells [28,33,37]. The suppression of NK activity therefore may be related to the increased incidence of tumors in HIV infected individuals. Moreover, there is evidence to suggest that this immune suppression is mediated by central mechanisms activated by the entry of HIV into the brain [31,40–42]. On the basis of these findings we propose that the central actions of HIV and subsequent neuroimmune interactions may be of particular importance in understanding the increase in tumor incidence associated with HIV infection.
A useful model for studying this proposal is based on the finding that some of the immunosuppressive effects of HIV can be produced by administration of the HIV glycoprotein, gp120, into the brain. Entry of gp120 into the brain, which typically occurs soon after infection with HIV [30], depresses the activity of NK cells and lymphocyte responses to mitogen stimulation [41]. The immunosuppressive effects of gp120 are thought to be mediated, at least in part, by centrally released interleukin-1 (IL-1) since entry of gp120 into the brain induces IL-1 secretion [31,41], and central administration of IL-1β suppresses the activity of NK cells, the response of lymphocytes to mitogen stimulation, and interleukin-2 production by splenic and blood lymphocytes [40–42,46]. Furthermore, co-infusion of IL-1β with α-melanocyte-stimulating hormone (α-MSH), which blocks many of the biological actions of IL-1 [25], blocks the IL-1-induced suppression of NK cell activity [42,46]. Finally, there is evidence to suggest that IL-1 is involved in the regulation of metastasis and growth of tumors [26].

Systemic administration of neutralizing antibodies to IL-1, or receptor blockade, reduces the number and size of experimentally-induced lung and liver metastatic melanomas in mice [9,43], whilst administration of IL-1 to mice with subcutaneous melanomas significantly increases the size of the melanoma [26]. On the basis of these findings we hypothesized that gp120-induction of IL-1 secretion in the brain may account, at least in part, for the increased incidence and aggressiveness of tumors in HIV-infected individuals.

In this research we assessed the effect of gp120 on tumor progression and the role of IL-1 in mediating these effects. We employed a mammary adenocarcinoma cell line, MADB106, which is syngeneic to the Fischer 344 (F344) rats used in these studies. MADB106 cells were developed to metastasize only to the lungs following intravenous (i.v.) inoculation [2]. Pulmonary retention of MADB106 tumor cells, and the subsequent growth of lung metastases, have been shown to be tightly regulated by NK cell activity during the first 24 h after tumor inoculation [2,3]. During this initial post-inoculation period, there is a marked NK-dependent destruction of MADB106 tumor cells, and lung clearance of these tumor cells is highly predictive of the number of lung tumors that can be observed 4 weeks after inoculation. Hence, lung clearance of MADB106 tumor cells provides a short-term index of resistance to metastasis, a process highly dependent on NK activity, whilst lung colonization assesses long-term metastatic outcome. This model also allows us to make predictions about the impact of gp120 on NK activity since both of these outcome measures (i.e., lung clearance and lung colonization) have been shown to reliably reflect NK cell activity in the live animal [2–5,49].

In Expt. 1A and 1B we examined the effect of intracerebroventricular (i.c.v.) administration of gp120 on the metastasis and lung clearance of MADB106 tumor cells. In Expts. 2–4 we tested the hypothesis that the effects of gp120 on metastasis and lung clearance are mediated by centrally released IL-1.

### 2. Methods and materials

#### 2.1. Subjects

Male F344 rats, 80 days old, were obtained from Charles River Breeding Laboratories (Wilmington, MA, USA). They were housed individually in a single colony room on a 12-h light cycle with food and water available ad libitum until commencement of the experiment. All animals were habituated to handling for at least one week prior to the commencement of each experiment.

#### 2.2. Cannulation and infusion procedure

Animals were anaesthetized with sodium pentobarbital (50 mg/kg, IP) and positioned in a stereotaxic instrument. A burr hole was drilled 1 mm posterior to bregma and 1.5 mm lateral to the midline and a 26-gauge guide cannula (Plastics One, Roanoke, VA), was lowered 4 mm below the skull surface [29]. The tip was positioned 1 mm above the lateral ventricle. The guide cannula was secured to the skull with three stainless steel screws and dental cement. A 33-gauge wire attached to a cap was used to seal the guide cannula. After the operation, rats were habituated daily to the i.c.v. injection procedure. On the day of the experiment, the solutions were injected through a 33-gauge cannula inserted into the guide cannula. The injection cannula was connected to a microsyringe pump by PE-50 tubing, and solutions were administered at a rate of 10 μl/min. At the completion of the experiment, brains were removed and histological examination was used to confirm correct placement of the cannula. Animals in which faulty cannula placement was observed were removed from the analysis.

#### 2.3. Biochemicals

Gp120 (Bartels Issaquah, WA, USA) was infused at a concentration of 4 μg/10 μl phosphate buffered saline (PBS). α-MSH (Sigma, St. Louis, MO, USA) was infused at a concentration of 20 ng/10 μl PBS. Recombinant human IL-1β (Biological Response Modifiers Program, NCI, USA) was infused at a concentration of 20 ng/10 μl PBS. The IL-1 ra (AMGEN, Boulder, Colorado, USA) was infused at a concentration of 50 μg/10 μl PBS.

#### 2.4. Tumor cells: maintenance and radiolabeling

The MADB106 tumor cell is a selected variant cell line obtained from a pulmonary metastasis of a mammary adenocarcinoma chemically induced in the F344 rat [2]. Following i.v. administration, this tumor cell, which is...
syngeneic to the F344 rat, reliably colonizes to the lungs, forming well-defined surface metastases by 4 weeks post inoculation. Cells were maintained in 5% CO$_2$ at 37°C in monolayer cultures. They were grown in complete medium: RPMI 1640 media (Gibco, Grand Island, NY) supplemented with 10% heat-inactivated fetal bovine serum, l-glutamine (2 mM), non-essential amino acids (0.1 mM), sodium pyruvate (1 mM), and Gentamycin (0.01 mg/ml). Cells were trypsinized (0.25%) to remove from the flask. For DNA labeling, 0.4 µCi $^{125}$IDUR (ICN Chemicals, Irvine, CA) per ml complete medium was added to the cell culture two days prior to harvesting. Prior to use, cells were separated from the flask, washed twice, and resuspended in PBS.

2.5. Assessment of tumor metastases and lung clearance of radiolabeled cells

Two hours after i.c.v. infusions, rats were lightly anesthetized with halothane and $1 \times 10^5$ tumor cells in 0.5 ml PBS were injected into the tail vein. Animals were returned to their home cages and 2 h later were lightly anaesthetized with halothane and inoculated via the tail vein with MADB106 tumor cells ($1 \times 10^5$/0.5 ml PBS). Four weeks after inoculation animals were euthanized with halothane and lungs were obtained for enumeration of tumor metastases.

3. Experimental procedures

3.1. Expt. 1A: Effect of i.c.v. administration of gp120 on the metastasis of MADB106 tumor cells

One week after cannula implantation surgery, animals were randomly divided into two groups: gp120 ($n = 25$) and PBS ($n = 15$). One group of animals (gp120) was infused i.c.v. ($10 \mu l$/min) with gp120 (4 µg gp120/10 µl PBS), the other group was infused with an equivalent volume of the vehicle (PBS). Animals were returned to their home cages and 2 h later were lightly anaesthetized with halothane and inoculated via the tail vein with MADB106 tumor cells ($1 \times 10^5$/0.5 ml PBS). Four weeks after inoculation animals were euthanized with halothane and lungs were obtained for enumeration of tumor metastases.

3.2. Expt. 1B: Effect of i.c.v. administration of gp120 on lung clearance of MADB106 tumor cells

One week after cannula surgery animals were randomly divided into two groups ($n = 15$/group). One group of animals (gp120) was infused i.c.v. (10 µl/min) with gp120 (4 µg/10 µl PBS), the other group (PBS) was infused with an equivalent volume of the vehicle. Animals were returned to their home cages and 2 h later were lightly anaesthetized with halothane and inoculated via the tail vein with radiolabeled $^{125}$IDUR MADB106 tumor cells ($1 \times 10^5$/0.5 ml PBS). Six hours later, animals were euthanized with halothane and lungs were obtained for assessment of radioactivity.

3.3. Expt. 2: Effect of i.c.v. administration of IL-1β on lung clearance of MADB106 tumor cells

The same procedure as in Expt. 1B was used to examine the effects of IL-1β on lung clearance except that one group of animals (IL-1β) was infused with IL-1β (10 ng/10 µl), and a second group (PBS) was infused with the equivalent volume of the vehicle, PBS ($n = 10$/group). Two hours later, the animals were lightly anaesthetized with halothane and inoculated via the tail vein with radiolabeled $^{125}$IDUR MADB106 tumor cells ($1 \times 10^5$/0.5 ml PBS). Six hours later, animals were euthanized with halothane and lungs were obtained for assessment of radioactivity.

3.4. Expt. 3A: Effect of i.c.v. co-administration of gp120 and α-MSH on lung clearance of MADB106 tumor cells

One week after cannula implantation surgery animals were randomly divided into four groups ($n = 8$/group): PBS/PBS, PBS/gp120, α-MSH/PBS, α-MSH/gp120. Animals were infused i.c.v. with either α-MSH (20 ng/10 µl PBS), or vehicle (10 µl/PBS), followed by a second infusion of either gp120 (4 µg/10 µl PBS), or vehicle (10 µl PBS). Animals were returned to their home cages and 2 h later, were lightly anaesthetized with halothane and inoculated via the tail vein with radiolabeled $^{125}$IDUR MADB106 tumor cells ($1 \times 10^5$/0.5 ml PBS). Six hours later, animals were euthanized and lungs were obtained for assessment of radioactivity.
3.5. Expt. 3B: Effect of i.c.v. co-administration of gp120 and IL-1 ra on lung clearance of MADB106 tumor cells

To examine the effect of IL-1 ra on lung clearance of tumor cells animals were cannulated as described above, and 1 week later were randomly divided into four groups (n = 10/group): PBS/PBS, PBS/gp120, IL-1 ra/PBS, IL-1 ra/gp120. Animals were infused i.c.v. with either IL-1 ra (50 μg/10 μl PBS), or vehicle (10 μl/PBS), followed by a second infusion of either gp120 (4 μg/10 μl PBS), or vehicle (10 μl PBS). Animals were returned to their home cages and 2 h later were lightly anaesthetized with halothane and inoculated via the tail vein with radiolabeled [125I]IDUR MADB106 tumor cells (1 x 10^5/0.5 ml PBS). Six hours later, animals were euthanized and lungs were obtained for assessment of radioactivity.

3.6. Expt. 4: Effect of systemic administration of gp120 or IL-1β on lung clearance of MADB106 tumor cells

One week after cannula implantation surgery animals were randomly divided into three groups (n = 7/group): PBS, gp120, or IL-1β. Animals were infused i.v. with either gp120 (4 μg/0.5 ml PBS), IL-1β (10 ng/0.5 ml PBS), or vehicle (0.5 ml PBS). Animals were returned to their home cages and 2 h later were lightly anaesthetized with halothane and inoculated via the tail vein with radiolabeled [125I]IDUR MADB106 tumor cells (1 x 10^5/0.5 ml PBS). Six hours later, animals were euthanized and lungs were obtained for assessment of radioactivity.

3.7. Statistical analysis

Data analysis was carried out using the Minitab statistical computing system (Minitab, State College, PA). Mean values were compared and statistical significance was assessed using appropriate analyses of variance (ANOVA) and t-tests. Pairwise comparisons were carried out using the Bonferroni procedure for a priori comparisons.

4. Results

4.1. Expt. 1A and 1B: Effect of i.c.v. administration of gp120 on the metastasis and lung clearance of MADB106 tumor cells

Fig. 1 illustrates the mean number of lung tumors in animals injected i.c.v. with either gp120 or the vehicle (PBS) prior to inoculation with MADB106 tumor cells. Data analysis revealed that treatment with gp120 significantly increased the number of lung metastases [t(35) = 2.63, p < 0.02] compared to treatment with the vehicle.

4.2. Expt. 2: effect of i.c.v. administration of IL-1β on lung clearance of MADB106 tumor cells

Fig. 2 illustrates the percentage of radioactivity retained in the lungs of animals injected i.c.v. with either gp120 or the vehicle (PBS) prior to inoculation with radiolabeled MADB106 tumor cells. Treatment with gp120 was found to significantly increase the retention of labeled tumor cells in the lungs [t(27) = 1.75, p < 0.05] when compared to the PBS control group.
to significantly increase the retention of tumor cells in the lungs compared to vehicle treated animals \( t(14) = 2.45, p = 0.028 \).

4.3. Expt. 3A and 3B: effect of i.c.v. co-administration of gp120 and either \( \alpha \)-MSH or IL-1ra on lung clearance of MADB106 tumor cells

Fig. 4 illustrates the effect of co-administration of gp120 with \( \alpha \)-MSH on lung clearance of radiolabeled MADB106 tumor cells. A two-way ANOVA indicated a significant interaction between the first (\( \alpha \)-MSH/PBS) and second injection (PBS/gp120) \( F_{1,24} = 6.071, \ p = 0.02 \). Bonferroni comparisons indicated that gp120 significantly \( p < 0.05 \) increased retention of MADB106 tumor cells, and this was blocked by co-infusion of \( \alpha \)-MSH \( p < 0.01 \), and \( \alpha \)-MSH had no effect itself. Fig. 5 illustrates the effect of co-administration of gp120 with IL-1 ra on lung clearance of radiolabeled MADB106 tumor cells. A two-way ANOVA indicated a significant interaction between the first (IL-1 ra/PBS) and second injection (PBS/gp120) \( F_{1,21} = 3.741, \ p = 0.02 \). Bonferroni comparisons indicate that gp120 significantly \( p < 0.01 \) increased retention of MADB106 tumor cells in the lungs, and this was blocked by co-infusion of IL-1 ra \( p < 0.01 \). IL-1 ra had no effect itself.

4.4. Expt. 4: effect of systemic administration of gp120 or IL-1B on lung clearance of MADB106 tumor cells

Data analysis revealed there were no significant differences between the three groups. There was no effect of either gp120 or IL-1B on lung clearance of labeled MADB106 tumor cells when compared to vehicle-treated controls.

5. Discussion

The present study demonstrates that decreased lung clearance and enhanced lung colonization of MADB106
tumor cells is mediated by gp120-stimulated centrally released IL-1. Three lines of evidence are provided to support this conclusion: (1) Intracerebral infusion of the HIV gp120, decreased lung clearance and enhanced metastatic colonization of MADB106 tumor cells. (2) The i.c.v. infusion of IL-1β also inhibited lung clearance of MADB106 tumor cells, and 3) co-infusion of gp120 with α-MSH or the IL-1 receptor antagonist blocked the gp120-induced suppression of lung tumor cell clearance. These findings are of particular significance since they are the first to demonstrate, in a live animal model, a positive relationship between entry of the HIV gp120 into the brain and tumor metastasis. Furthermore, they provide the first in vivo evidence that this effect is mediated by centrally released IL-1. Given that lung clearance and metastasis of MADB106 tumor cells have been shown to reflect NK activity in vivo [2–5,49] these findings also indicate that gp120-induced central secretion of IL-1 may enhance tumor metastasis via inhibition of NK activity in the periphery.

Previous studies have demonstrated that gp120 suppresses NK activity. However, in these studies, NK activity was assessed using standard in vitro methods for assessing NK cell activity [41]. The MADB106 tumor cell model used in this research has the advantage of allowing us to examine tumor progression and NK activity in vivo. Colonization of the lungs is tightly regulated by the activity of NK cells, the immune cell population primarily engaged in surveillance of MADB106 tumor cells in the early post inoculation period [2]. The two outcome measures used in this study, i.e., lung clearance and tumor colonization, have been shown to reliably reflect NK cell activity in vivo [2–5,49]. The findings of this study therefore indicate that entry of gp120 into the brain most likely promotes tumor metastasis via the suppression of NK-dependent clearance of tumor cells within a few hours post-inoculation. In addition to their direct cytotoxic actions NK cells also affect tumor progression via their interaction with T cell-mediated mechanisms [13,47]. The NK cell is critical in determining whether antigen stimulated CD8+ cells proliferate or differentiate into cytotically active effector cytotoxic T lymphocytes (CTLs), and there is recent evidence to suggest that T cell mechanisms play a role in regulation of MADB106 metastasis in the later post-inoculation stages [16]. This impaired surveillance by NK cells may be one mechanism to account for the increased incidence of tumors, especially NK sensitive tumors, in HIV-infected individuals.

The findings of the present research also demonstrate that centrally administered IL-1β facilitates tumor metastasis, suggesting that the gp120-induced promotion of tumor metastases is mediated by centrally released IL-1. This finding is consistent with previous reports demonstrating that gp120 induces IL-1 production in the brain [27,31,41,44], and that centrally administered IL-1β suppresses several immune functions, including the activity of NK cells [21,40,42]. In this study, co-administration of α-MSH or IL-1 ra prevented the gp120-induced increase in tumor retention. These findings are consistent with previous studies, which demonstrated that α-MSH can block many of the biological and immunological effects of IL-1 [25], including the suppression of NK cell activity [42,46] lymphocyte responses to mitogenic stimulation, and IL-2 production [40]. Evidence that the actions of IL-1 are centrally mediated and are not due to leakage from the brain ventricular system to the periphery has previously been provided by the demonstration that IL-1β administered intraperitoneally does not suppress NK cell activity [45]. In this study we also report that systemic administration of either gp120 or IL-1, at doses equivalent to the i.c.v. doses, does not influence the lung retention of MADB106 tumor cells. Taken together these findings suggest that gp120-induced promotion of tumor metastasis is mediated by centrally released IL-1.

Several mechanisms may be involved in mediating the effects of centrally released IL-1 on immunity and metastasis. IL-1, for instance, is a growth factor for some, but not all tumor cell lines [23]. In many cases this growth promoting effect is due to IL-1-induced synthesis of other cytokines, in particular interleukin-6 (IL-6) [23]. IL-6 is a cytokine known to act directly on certain malignant tumor cell lines as a growth factor [12]. There is neither evidence to date as to whether IL-1 or IL-6 have direct proliferative effects on MADB106 tumor cells, nor can such explanations account for the acute effects of IL-1 on lung clearance reported here. Of particular relevance, however, is the fact that centrally administered IL-1 induces the release of IL-6 which triggers plasma protein synthesis by hepatocytes during the acute phase response that occurs in response to injury, infection, or neoplasia [48]. One of the proteins that increases acutely is fibrinogen. Deposition of fibrinogen in tissues enhances leukocyte migration and retention in extravascular tissues [39]. Given that tumor cells use the same mechanisms as immune cells to migrate into the tissues, conditions that facilitate cell migration are likely to increase the probability that tumor cells migrate successfully to new sites. Assessment of acute phase proteins, and their effect on tumor cell metastasis could be a useful outcome measure in future studies.

It has also been proposed that IL-1 suppresses peripheral immune responses by activating the hypothalamic–pituitary–adrenal (HPA) axis [21,34,40]. IL-1 release in the brain stimulates the secretion of adrenocorticotropic hormone (ACTH) and elevates plasma cortisol levels [7,8] which have been shown, in some instances, to be immunosuppressive [14,17,24,35,38]. Moreover, co-infusion of IL-1β with α-MSH blocks the IL-1-β-induced increase in plasma ACTH and cortisol and the suppression of NK cell activity [45,46]. There is, however, evidence for mediation of IL-1’s effects by pathways independent of the HPA axis, since IL-1-induced immunosuppression was demonstrated in adrenalectomized ani-
mals in the absence of adrenal hormones [42]. A neural pathway has been implicated given that blocking neural transmission at sympathetic ganglia partially attenuates the IL-1-induced suppression of NK cell activity [45]. The sympathetic nervous system seems to be of particular relevance given that centrally administered IL-1 increases splenic sympathetic activity [18]. Electrical stimulation of the splenic nerve also reduces NK cell activity, and this effect is blocked by pretreatment with β-receptor antagonists [22]. Furthermore, activation of the β-2 adrenergic receptors mediates the suppressive effects of acute stress on NK cell activity and results in increased metastatic spread of MADB106 tumor cells in the rat [6]. Future research should address the role of neural and hormonal mechanisms in mediating the promotion of tumor metastasis by gp120.

6. Conclusion

In conclusion, the present study demonstrates that entry of the HIV gp120 into the brain enhances tumor metastasis and this appears to be mediated by the central release of IL-1. The inhibition of lung clearance and enhancement of tumor metastasis by gp120 is most likely mediated by the suppression of NK- and T cell-mediated immunity in the periphery, possibly via IL-1-induced activation of the HPA axis and the sympathetic nervous system. These findings may provide a mechanism to account for the finding that HIV infection is associated with a high incidence of malignancy. Although significant progress is being made with aggressive combined chemotherapy in the treatment of AIDS, in cases where the disease is complicated by the presence of malignancy, the prognosis remains exceptionally poor [32,36]. Treatment options are limited because the poor immunological status of AIDS patients renders them intolerant to standard anti-tumor therapy [10]. Thus, there is a need to develop new approaches for effective treatment of HIV-related malignancies. Attenuation of the immunosuppressive and tumor enhancing effects of gp120 by selective pharmacological blockade of the central effects of IL-1 may provide such an approach.

Acknowledgements

This research was supported by The Norman Cousins Program in Psychoneuroimmunology at UCLA (D.M.H.), Department of Veterans Affairs Medical Research Service, NIH/NIAAA AA09850, and the US–Israel Binaional Science Foundation 94-00062 (R.Y and A.N.T). We also acknowledge the generous donation of IL-1 from the National Cancer Institute (Biological Response Modifiers Program, NIH/NCI) and IL-1 receptor antagonist from AMGEN (Boulder, CO, USA). The expert technical assistance of Mr. Ng Heng and Khai Nguyen was also greatly appreciated.

References


[43] F. Vidal-Vanaclocha, C. Amezaga, A. Asumendi, G. Kaplanski, C.A. Dinarello, Interleukin-1 receptor blockade reduces the number and size of murine B 16 melanoma hepatic metastases, Cancer Res. 54 (1994) 2667–2672.


