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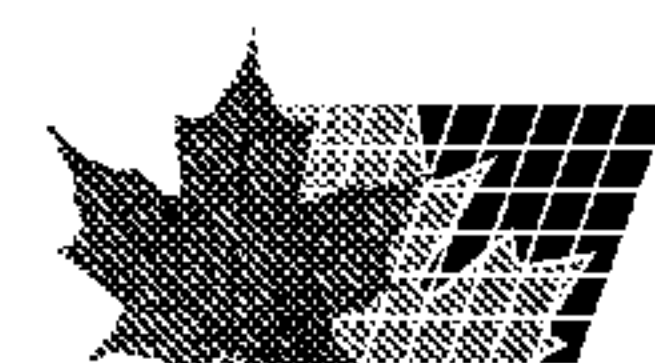
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(54) Titre : AMELIORATION DE L'IMMUNITE CONTRE LE CANCER PAR L'INTERMEDIAIRE DE L'INHIBITION DE
L'ARGINASE

(54) Title: ENHANCEMENT OF ANTICANCER IMMUNITY THROUGH INHIBITION OF ARGINASE

(57) **Abrégé/Abstract:**

Strategies are disclosed for generating dendritic cells that possess immune stimulatory activity despite immune suppressive factors elaborated by the tumor. An aspect of the invention includes blocking the arginase pathway in dendritic cells that are pulsed with tumor antigens. Alternatively, dendritic cells modified according to the invention are placed proximally near tumor tissues so as to naturally acquire tumor antigens and stimulate T cell responses. An object of this invention is to allow stimulation of Th1 immunity in cancer-bearing mammals that generally are predisposed to Th2 or anergic responses.



Abstract of the Disclosure

Strategies are disclosed for generating dendritic cells that possess immune stimulatory activity despite immune suppressive factors elaborated by the tumor. An aspect of the invention includes blocking the arginase pathway in dendritic cells that are pulsed with tumor antigens. Alternatively, dendritic cells modified according to the invention are placed proximally near tumor tissues so as to naturally acquire tumor antigens and stimulate T cell responses. An object of this invention is to allow stimulation of Th1 immunity in cancer-bearing mammals that generally are predisposed to Th2 or anergic responses.

DESCRIPTION

Field of the Invention

The invention disclosed relates to the field of methods of treating cancers. More specifically, the invention pertains to the field of cancer immunotherapy.

Background of Invention

It is established that tumor cells evade the immune system of the patient. This contributes to the general failure of immunotherapeutic approaches [1-3]. Secretion of soluble factors by tumors, such as IL-10 [4-6], free TGF- β [7], TGF- β bound to immunoglobulin [8], prostaglandins [9], low molecular weight retroviral p15E-like factors [10], immunosuppressive acidic protein [11], soluble MUC-1 [12], and tumor-shed gangliosides [13] contribute to suppression of various aspects of the immune responses. Tumors also protect themselves from immune attack by coercing cells of the immune system to inhibit other cells of the immune system.

CD4 T cells have the ability to differentiate along the Th1 or Th2 pathway, which in turn stimulate cell-mediated, or antibody-mediated immune responses [14]. In order to protect the host from autoimmunity, CD4 T cells also possess the ability to differentiate into T regulatory (Treg) cells that have the ability to inhibit activated T cells regardless whether they are Th1 or Th2 [15]. A specific type of Treg possesses the phenotype of CD4⁺ CD25⁺. These cells are critical for protection against autoimmunity, as demonstrated by experiments in which depletion of this cell population resulted in accelerated diabetes [16], or organ-specific autoimmune diseases [17]. Furthermore, transfer of Treg cells into animals prone to autoimmunity can delay progression or outright block disease occurrence [18].

Thus while Treg cells naturally possess the function of protecting the body against harmful autoimmune attacks, it is these same Treg cells that cancer uses to further shield itself from immune attack. For example, as stated above, tumor cells secrete various immune-inhibitory factors. These factors possess the property of not only inhibiting

immune attack, but also programming the differentiation of T cells into Treg cells. CD4⁺ CD25⁺ Treg cells have been found to circulate in higher numbers in cancer patients, where they inhibit T cell and NK cell responses [19]. Depletion of Treg cells allows for greater efficacy of cancer-specific vaccines in murine models [20, 21].

Another type of immunological cell that cancer subverts for its own means is the macrophage. Classically, macrophages are known to possess anti-cancer properties [22], and have been described to spontaneously lyse or arrest proliferation of tumor cells [23, 24]. However evidence is accumulating the macrophages can actually be involved in the stimulation of tumor growth. One suggestion of this was the observation that mice lacking mature macrophages (op/op mice) due to congenital absence of M-CSF are actually more resistant to growth of implanted tumors when compared to wild-type mice [25]. Further investigation found that cancer patients possessing high number of macrophages infiltrating the tumor had a poorer prognosis than patients with low numbers of tumor-infiltrating macrophages [26]. It was thought the mechanism by which the tumor-infiltrating macrophages stimulated an aggressive cancer phenotype was through increasing angiogenesis via secretion of endothelial-promoting factors such as VEGF [27].

To parallel the example with Treg cells, macrophages with immune inhibiting function, also termed M-2 or alternatively activated macrophages, are found in the normal life of a mammal. The normal function of these alternatively activated macrophages is thought to be promotion of wound healing [28]. Alternatively activated macrophages possess distinct molecular markers such as the genes *Ym1* and *Fizz1* [29]. However, as is the case for Treg, tumors can induce alternatively activated macrophages in the tumor environment [30]. These cells inhibit local immune responses, and are themselves involved in the generation of Treg cells [31].

Tumors themselves and alternatively activated macrophages express high levels of the arginase, an enzyme that breaks down arginine into L-ornithine and urea [32, 33]. L-ornithine is further degraded into polyamines such as spermine and spermidine by the

enzyme ornithine decarboxylase. Immune suppression induced by arginase overexpression can occur through several pathways: a) arginine-depletion by the tumor results in destruction of important T cell signaling molecules such as the TCR-zeta chain [34]; b) polyamines suppress T cell activation [35]; c) polyamines suppress the proinflammatory function of macrophages [36]; and d) polyamines suppress activity of NK cells [37].

Macrophages from prototypical Th1 strains (C57BL/6, B10D2) are more easily activated to produce NO with either IFN-gamma or LPS than macrophages from Th2 strains (BALB/c, DBA/2). In marked contrast, LPS stimulates Th2, but not Th1, macrophages to increase arginine metabolism to ornithine [38]. In the context of tumors, it is known that Th2 cytokines predispose to increased tumor aggressiveness and promote immune evasion. Th2 cytokines are also known to stimulate arginase production. In macrophages incubated with T cells: Th1 T cells lead to an exclusive induction of iNOS, whereas Th2 T cells up-regulated macrophage arginase without inducing iNOS. Ab blocking experiments revealed the critical importance of IL-4 and IL-10 for arginase up-regulation [39]. Furthermore, the component of macrophage supernatant that stimulated tumor growth was substantially correlated with arginase [40].

As stated above, a characteristic feature of tumors is high production of the enzyme arginase. Serum arginase is a good marker for colorectal carcinoma progression [41-43]. Values of serum arginase activity in patients with breast carcinoma were up to 4-fold higher than those found in healthy women [44]. In prostate cancer tissue arginase expression correlates with severity of disease. A study evaluating arginase activities in prostate tissues of 50 patients with benign prostatic hyperplasia and in 23 patients with prostatic carcinoma found that arginase was elevated in cancer tissues as compared to benign prostatic hyperplasia (fivefold; $P < .001$). A positive correlation between arginase activity and Gleason grade of the tumors was also found [45].

Although tumor-derived arginase and macrophage arginase are believed to be associated with immune suppression in cancer, a substantial question remains: tumors themselves

are poor T cell stimulators, similarly macrophages can stimulate T cells but are not optimal, since dendritic cells (DC) are the most potent stimulators of T cells, do they express arginase? Is tumor-induced arginase expression a mechanism by which cancer cells block T cell activation?

Although inhibitors of arginase [46], and inhibitors of enzymes downstream of arginase can block tumor growth and progression, the effects of these inhibitors is still therapeutically limited. The present invention is based on our findings that 1) DC expression of arginase causes inhibition of immune function, and 2) reversal of arginase expression on DC can over-come the inhibitory effects of the tumor on DC function, those stimulating antitumor immunity.

This background information is provided for the purpose of making known information believed by the applicant to be of possible relevance to the present invention. No admission is necessarily intended, nor should be construed, that any of the proceeding information constitutes prior art against the present invention.

Description of the Invention

Disclosed are methods rendering dendritic cells potent stimulators of immune activation in such a manner that tumor-secreted immune suppressants do not inhibit their function. A specific aspect of the invention relates to treating dendritic cells with a pharmacological inhibitor of the enzyme arginase such as N(omega)-hydroxy-L-arginine or N(omega)-hydroxy-nor-L-arginine. This treatment would inhibit the ability of the dendritic cell to react to a variety of tumor-secreted immune suppressants, in particular cytokines such as IL-10. In the same spirit, the dendritic cell may be modulated through the use of other known inhibitors that block the function of the enzyme arginase. Said inhibitors may be small molecular peptides, chemical analogues or suicide substrates.

In another aspect, dendritic cell expression of the enzyme arginase is suppressed using known genetic inhibitory techniques such as siRNA, antisense oligonucleotides,

hammerhead ribozymes or morpholinos. The suppression of arginase may be performed in an ex vivo manner, wherein the dendritic cells, or their progenitors are extracted from the blood or bone marrow of the patient, cultured and transfected outside of the body, and subsequently reintroduced into the patient in such a manner as to stimulate immune response against the tumor. In this aspect, the dendritic cells are either pulsed with tumor-derived antigen, or alternatively, are allowed to naturally engulf tumor antigens from the tumor itself if the dendritic cells are injected intratumorally.

Another aspect of the invention is that dendritic cells whose ornithine decarboxylase is inhibited can be used for stimulating anti-tumor responses by virtue of the fact that they are not prone to tumor-induced immune suppression. Both chemical and/or genetic (ie siRNA, antisense oligonucleotides, hammerhead ribozymes or morpholinos) inhibition of ornithine decarboxylase will cause the formation of potent immune stimulatory dendritic cells that can prime anti-tumor responses more effectively than unmanipulated dendritic cells.

Another aspect of the invention is that inhibition of both arginase and ornithine decarboxylase endow the dendritic cell with protection from tumor-induced apoptosis. Inhibition of expression and/or function of arginase and/or ornithine decarboxylase allows for more effective immune stimulation by increasing the amount of time that a dendritic cell can live in the context of the tumor-secreted, and surface bound apoptotic factors.

Another aspect of the invention is that manipulation of arginase and ornithine activity in dendritic cells can be achieved by systemic manipulation through dietary restriction or through systemically administered drugs such as DFMO. While this approach may be less efficacious than ex vivo manipulation, it is more convenient in situations where ex vivo culture and manipulation of dendritic cells is impossible.

Description of Figures

Figure 1 illustrates that arginase inhibition blocks IL-10-induced suppression of DC IL-12 production.

Figure 2 illustrates that arginase inhibition renders DC resistant to IL-10 mediated suppression of allostimulatory capacity.

Figure 3 illustrates that arginase inhibition blocks tumor supernatant-induced suppression of DC IL-12 production.

Figure 4 illustrates that arginase inhibition renders DC resistant to tumor supernatant mediated suppression of allostimulatory capacity.

Figure 5 illustrates that arginase inhibition renders DC resistant to tumor supernatant-induced apoptosis.

Figure 6 illustrates that ODC inhibition renders DC resistant to tumor supernatant-mediated suppression of IL-12 production.

Figure 7 illustrates that ODC inhibition renders DC resistant to tumor supernatant-mediated suppression of allostimulatory function.

Figure 8 illustrates that ODC inhibition renders DC resistant to tumor supernatant-induced apoptosis.

Figure 9 illustrates that arginase and ODC-inhibited DC, but not untreated DC, are able to induce tumor suppression in vivo.

Detailed Description of Invention

One embodiment of the disclosed invention teaches methods of preparing a cancer vaccine using dendritic cells that are resistant to cancer-induced immune suppression. As stated in the Background section, the arginase pathway of metabolism is upregulated in cancer cells and cancer-secreted metabolites of this pathway are involved in immune suppression. However, part of the novelty of this invention resides in our findings that tumor-secreted immune suppressive factors, such as IL-10, are also potent inducers of the arginase pathway in dendritic cells. Since T cells are more potently stimulated or inhibited during their interaction with dendritic cells, as compared to their interaction with tumor cells, it would be more likely that the arginase upregulation in the dendritic cell is more important for tumor-immune suppression than arginase upregulation in the tumor itself. Furthermore, arginase pathway activation is involved in the induction of dendritic cell apoptosis. The ability of tumors to secrete cytokines such as IL-10 which upregulate arginase activity in dendritic cells, could be a mechanism by which tumor cells induce apoptosis of dendritic cells. Therefore the inhibition of the arginase pathway in tumor cells would not only increase the resistance of dendritic cells to tumor-secreted immune suppressive factors but also protect dendritic cells from apoptosis.

For manufacturing a cancer vaccine using dendritic cells, blockade of the arginase pathway could be achieved through: a) inhibition of arginase enzymatic activity, b) inhibition of arginase gene expression, c) inhibition of ornithine decarboxylase enzymatic activity, or d) inhibition of ornithine decarboxylase gene expression.

In one cancer vaccine embodiment, dendritic cells are generated from peripheral blood mononuclear cells purified by density gradient. Monocytic cells are purified by adherence to plastic and cultured for 7-days in GM-CSF and IL-4 at a concentration between 20-100ng/ml, preferentially, 50 ng/ml. A concentration of 200 μ Mol NOAH is added to the culture every time the cells are passaged. Tissue culture media is changed once every-two days. An alternative modification is that ornithine-decarboxylase can be inhibited in

the DC culture by addition of the ornithine-decarboxylase inhibitor DFMO. Although several concentrations are useful, 3mM seems ideal based on our studies. These arginase/ornithine decarboxylase-inhibited DC are subsequently injected intra-tumorally, preferably at a concentration of 2×10^9 cells. Based on the endogenous ability of DC to uptake tumor antigens, these cells provide an autologous "natural" vaccine that stimulates T cell responses against the tumor antigens.

Examples

Example 1: Generation of dendritic cells resistant to the tumor-secreted cytokine IL-10

This example illustrates the generation of dendritic cells that are resistant to the immune-inhibitory activities of IL-10 by pretreating said dendritic cells with the arginase inhibitor *N*-hydroxy-L-arginine (NOHA).

DC were generated from bone marrow progenitor cells as previously described [47]. Briefly, bone marrow cells were flushed from the femurs and tibias of C57BL/6 mice (Jackson Labs, Bar Harbor ME), washed and cultured in 24-well plates (2×10^6 cells/ml) in 2 ml of complete medium (RPMI-1640 supplemented with 2mM L-glutamine, 100 U/ml of penicillin, 100 μ g of streptomycin, 50 μ M 2-mercaptoethanol, and 10 % fetal calf serum (all from Gibco RBL)) supplemented with recombinant GM-CSF (10 ng/ml; Peprotech, Rocky Hill, NJ) and recombinant mouse IL-4 (10 ng/ml; Peprotech). All cultures were incubated at 37°C in 5% humidified CO₂. Non-adherent granulocytes were removed after 48 hours of culture and fresh medium was added.

4 experimental groups were used:

- a) Unmanipulated DC cultures
- b) DC cultured in the presence of IL-10
- c) DC cultured in the presence of arginase inhibition
- d) DC cultured with IL-10 and arginase inhibition

In group (b) IL-10 was added throughout the culture period at a final concentration of 50 ng/ml. In group (c) a 200 μ M concentration of the arginase inhibitor NOHA (Sigma-Aldrich, Rockville IL) was added for the whole culture period. In group (d), the combination IL-10 and the arginase inhibitor NOHA were added as described in groups (b and c).

After 7 days of culture >90% of the cells expressed the characteristic DC-specific marker CD11c as determined by FACS. DC were washed in phosphate buffered saline (PBS) and plated in 24-well plates at a concentration of 2×10^5 cells per well. Cells were activated for 48 hours with LPS (10 ng/ml, Sigma Aldrich, St Louis, MO) + TNF- α (10 ng/ml, Peprotech). Supernatants were harvested and production of the tumor-inhibitory and Th1-activating cytokine IL-12 was assessed by sandwich ELISA (R&D Systems, Minneapolis, MN). As illustrated in Figure 1, DC generated in absence of IL-10 or arginase inhibition possessed strong IL-12 producing ability (a). Addition of IL-10 in the culture media resulted in the inhibition of IL-12 production (b). Inhibition of arginase activity induced a slight increase in activity production of IL-12(c). Surprisingly, arginase inhibition spared the DC of IL-10-mediated downregulation of IL-12 production.

Additionally, mixed lymphocyte reaction (MLR) was performed to assess functional allostimulatory capacity of the DC in groups a-d. For MLR, T cells were purified from BALB/c splenocytes using nylon wool columns and were used as responders (1×10^6 /well). DC from groups a-d ($5-40 \times 10^3$, C57/BL6 origin) were used as stimulators. 72 hour MLR was performed and the cells were pulsed with 1 μ Ci [3 H]-thymidine for the last 18 hours. The cultures were harvested on to glass fiber filters (Wallac, Turku, Finland). Radioactivity was counted using a Wallac 1450 Microbeta liquid scintillation counter and the data were analyzed with UltraTerm 3 software. As seen in Figure 2, DC generated in the presence of IL-10 were poor stimulators of allogeneic T cell proliferation. DC raised in the presence of arginase inhibition possessed a similar allostimulatory capacity as DC raised under control conditions. When DC were

generated in the presence of IL-10 under conditions of arginase inhibition, the allostimulatory effects were preserved. This suggests that arginase inhibition can act at the level of the dendritic cell to protect from immune suppressive activities of IL-10.

Example 2: Arginase inhibition allows normal DC maturation despite presence of tumor-secreted inhibitory factors.

It is reported that generating DC in the presence of supernatants from a variety of tumors results in the production of immature DC with poor allostimulatory capacity. Although IL-10 is a known tumor-secreted factor responsible for the inhibition of DC maturation, other factors such as ceramides, gangliosides, TGF- β and prostaglandins have also been reported. We therefore assessed whether inhibition of arginase activity could render DC resistant to the inhibitory effects of tumor –supernatant in a manner similar to IL-10.

DC were generated as described in Example 1 with the modification that IL-10 was replaced with 20% volume of day-2 supernatant from the murine Lewis lung carcinoma (3LL) cell line (American Type Culture Collection, Manassas VA). Arginase inhibition was performed by supplementation of the cell culture media with a 200 μ M concentration of the arginase inhibitor NOHA as in Example 1.

Experimental groups consisted of:

- a) Unmanipulated DC cultures
- b) DC cultured in the presence of 20% 3LL supernatant
- c) DC cultured in the presence of arginase inhibition
- d) DC cultured with 20% 3LL supernatant and arginase inhibition

Using the experimental conditions of Example 1, it was demonstrated that arginase inhibition was able to block the 3LL-induced inhibition of IL-12 production from DC (Figure 3).

The conditions of Example 1 were also used to assess whether arginase inhibition could block 3LL-induced suppression of DC allostimulatory capacity. As seen in Figure 4, arginase inhibition was successful at preserving DC allostimulatory ability despite the presence of 3LL supernatant.

Example 3 Arginase inhibition protects DC from tumor-induced apoptosis

It is reported that supernatants from a variety of tumor cells can induce apoptosis in DC. A method of protecting the DC from such apoptosis would theoretically result in a more efficacious tumor vaccine, as well as protect the immune responsive ability of the cancer patient.

Dendritic cells were generated as described in Example 1. At day 7 of culture the cells were divided into 4 groups

- a) Control: media from the 3T3 fibroblast cell line was added.
- b) Arginase inhibition: a 200 μ M concentration of the arginase inhibitor NOHA was added.
- c) Tumor supernatant: B-16 supernatant was added at 20% of tissue culture volume.
- d) Arginase inhibition in the presence of tumor supernatant. Both b and c were added.

At day 9 apoptosis was assessed using Annexin-V staining and analyzed by flow cytometry. As indicated in Figure 5 apoptosis was induced by the presence of tumor-supernatant while arginase inhibition was able to reduce this effect.

Example 4 Inhibition of ornithine decarboxylase (ODC) protects DC from tumor-induced immune suppression

DC were cultured as described in Example 1. In some groups media was replaced with 80% fresh media and 20% volume of day-2 culture supernatant from the murine Lewis lung carcinoma (3LL) cell line.

Experimental groups consisted of:

- a) Unmanipulated DC cultures
- b) DC cultured in the presence of 20% 3LL supernatant
- c) DC cultured in the presence of ODC inhibition
- d) DC cultured with 20% 3LL supernatant and ODC inhibition

ODC inhibition was achieved by administration of 3 mM DL-alpha-difluoromethylornithine (DFMO) into the culture media for the whole culture period.

Using the experimental conditions of Example 1, it was demonstrated that ODC inhibition was able to block the 3LL-induced inhibition of IL-12 production from DC (Figure 6).

The conditions of Example 1 were also used to assess whether ODC inhibition could block 3LL-induced suppression of DC allostimulatory capacity. As seen in Figure 7, ODC inhibition was successful at preserving DC allostimulatory ability despite the presence of 3LL supernatant.

Example 5 ODC inhibition protects DC from tumor-induced apoptosis

Dendritic cells were generated as described in Example 1. At day 7 of culture the cells were divided into 4 groups

- a) Control: media from the 3T3 fibroblast cell line was added.
- b) ODC inhibition: a concentration of 3 mM DL-alpha-difluoromethylornithine (DFMO) was added to the culture media.
- c) Tumor supernatant: B-16 supernatant was added at 20% of tissue culture volume.
- d) ODC inhibition in the presence of tumor supernatant. Both b and c were added.

At day 9 apoptosis was assessed using Annexin-V staining and analyzed by flow cytometry. As indicated in Figure 8 apoptosis was induced by the presence of tumor-supernatant while arginase inhibition was able to reduce this effect.

Example 6 Arginase-inhibited and ODC-inhibited DC induce anti-tumor responses in vivo

Male 6-8 week old C57BL/6 mice were injected with RM-1 murine prostate tumor cells (20,000 cells/100 μ l) in the right flank. Tumor size was determined using Vernier caliper. DC (20,000/100 μ l) raised using the conditions below were co-injected with tumor cells or tumor cells were injected alone:

DC generated as described in Example 1 in and raised under the following conditions:

- a) Fed every other day control media as described in Example 1
- b) Arginase was inhibited by addition of 200 μ M NOAH
- c) ODC was inhibited by addition of 3 mM DMFO

5 mice/group were used. Tumor size was evaluated on day 20 was evaluated. As seen in Figure 9, tumor cells injected alone, or with control DC grew to a much greater extent than tumors injected with DC raised in the presence of arginase or ODC inhibition. As repetition of this experiment in nu/nu on C57BL6 backgrounds resulted in equivalent tumor growth in all groups (data not shown), we postulate that relieving the DC of tumor-induced immune suppression by arginase inhibition or ODC inhibition, is a valuable strategy for induction of antitumor responses.

The foregoing invention has been described in such a manner to enable one skilled in the art to practice it. However, it will be readily apparent to one skilled in the art that after reading the teachings of this invention certain changes and modifications may be made without departing from the spirit or scope of the appended claims. Furthermore, the examples illustrated are by no means binding but provide a guide for practicing certain embodiments and aspects of the invention disclosed.

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THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. A cancer vaccine comprised of;
 - a) a dendritic cell,
 - b) a tumor antigen,
 - c) an arginase inhibitor.
2. The vaccine of claim 1 wherein a dendritic cell encompasses a bone marrow-derived cell expressing substantial amounts of the surface marker CD11c, MHC II, CD80, and CD86.
3. The vaccine of claim 1 wherein said dendritic cell is purified from bone marrow cultures at 4-13 days of culture in interleukin-4 and granulocyte-monocyte colony stimulating factor.
4. The cancer vaccine of claim 1 wherein a tumor antigen is derived from molecules found on tumors but not found on healthy, non-tumorous tissue.
5. The cancer vaccine of claim 1 wherein tumour antigens comprise of MUC-1, MAGE, BAGE, PSA, PAP, tyrosinase, and CEA.
6. A dendritic cell whose function is not substantially inhibited by immune suppressive factors in the body of a cancer patient whereas said dendritic cell is treated with a dose of the arginase inhibitor L-NOAH effective to substantially inhibit the enzyme arginase.
7. The dendritic cell of claim 6 wherein arginase inhibition is performed by incubation of 200 μ M L-NOAH with said dendritic cell.
8. A dendritic cell whose function is not substantially inhibited by immune suppressive factors in the body of a cancer patient wherein said dendritic cell is transfected with short

interfering RNA oligonucleotides ranging from 19-25 base pairs that possess substantial homology with an exon from the gene encoding arginase.

9. A dendritic cell whose function is not substantially inhibited by immune suppressive factors in the body of a cancer patient wherein said dendritic cell is transfected with a plasmid encoding a sequence that when transcribed into RNA yields a hairpin loop that degrades intracellularly into short interfering RNA that possesses substantial homology with an exon from the gene encoding arginase.

10. The utility of the dendritic cell of claims 6-10 as a cancer vaccine, wherein said dendritic cell is administered a tumor antigen and subsequently introduced into the body of a mammal in need thereof at a concentration needed to stimulate tumor-specific immunity.

11. A cellular vaccine composition wherein the dendritic cell of claim 10 is admixed into a biocompatible sponge-like material and inserted into the proximity of a tumor.

12. The cellular vaccine composition of claim 11 wherein the sponge-like material is comprised of epsilon-caprolactone-co-L-lactide reinforced with knitted poly-L-lactide fabric PCLA.

13. The cellular vaccine composition of claim 11 wherein the sponge-like material is comprised mainly of gelatin.

14. The vaccine composition of claim 11 wherein the sponge-like material is comprised of polyglycolic acid.

15. A method of protecting dendritic cells from tumor-induced apoptosis through inhibition of arginase pathway.

16. A method of blocking tumor-induced DC suppression by administering an effective amount of an inhibitor of ODC such as α -difluoromethylornithine (DFMO).

17. A method of blocking tumor-induced DC suppression by restricting arginine or polyamine intake.

18. A method of blocking tumor-induced DC suppression by administering drugs whose primary or secondary effects include suppression of arginine levels, arginase activity, or ODC activity or levels.

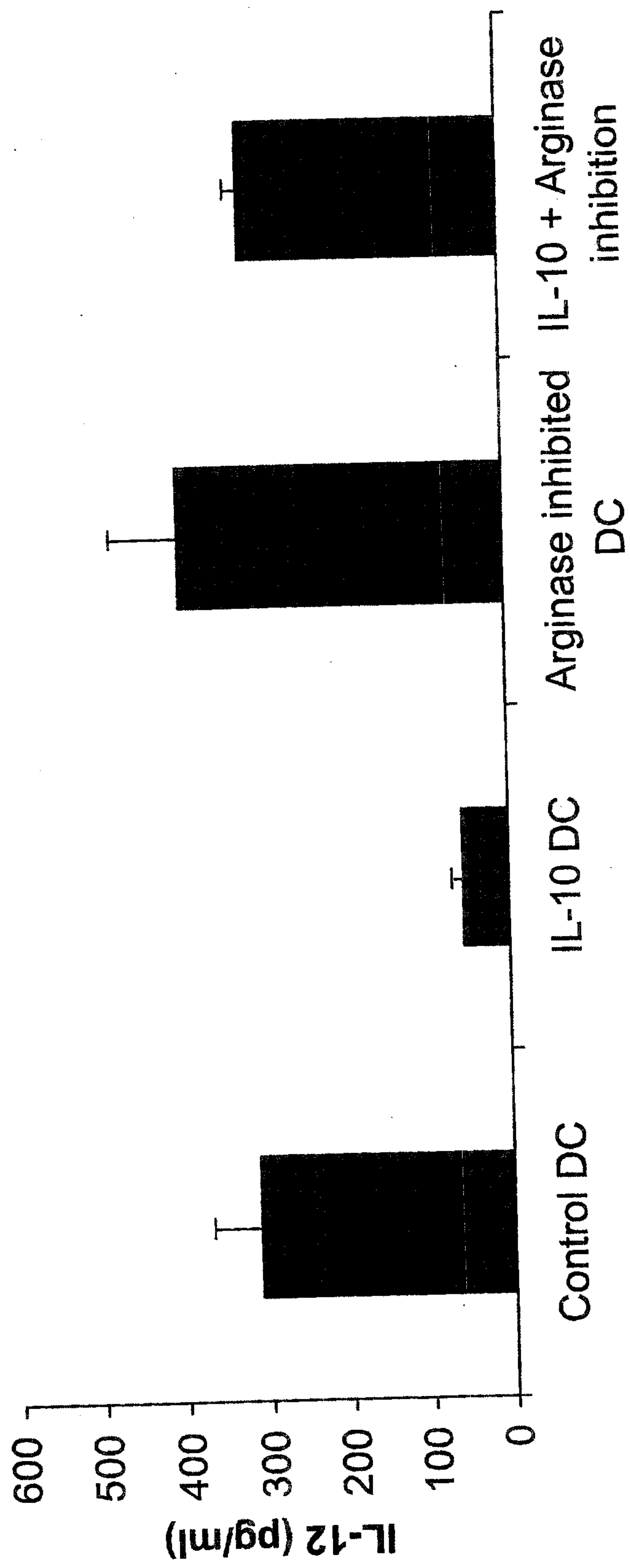
Figure 1

Figure 2

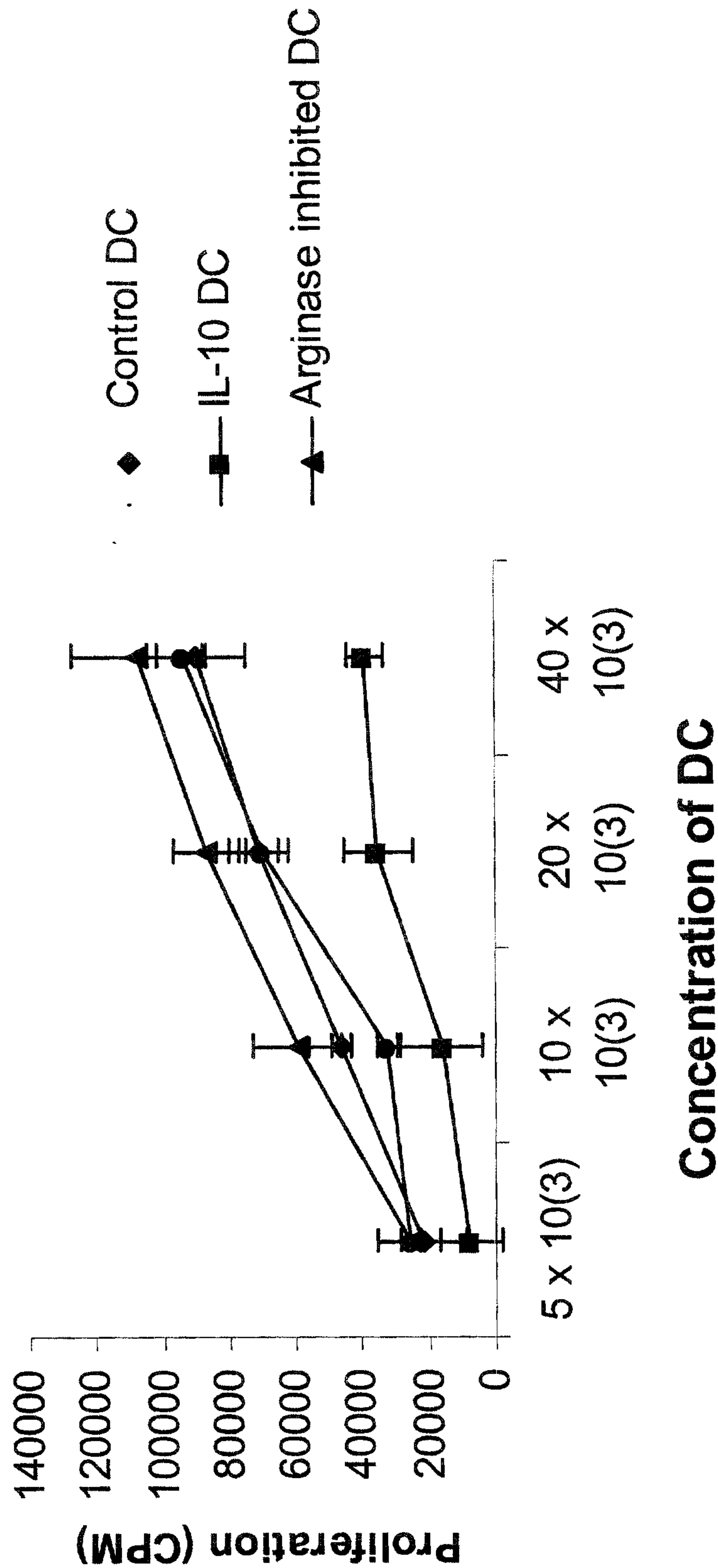


Figure 3

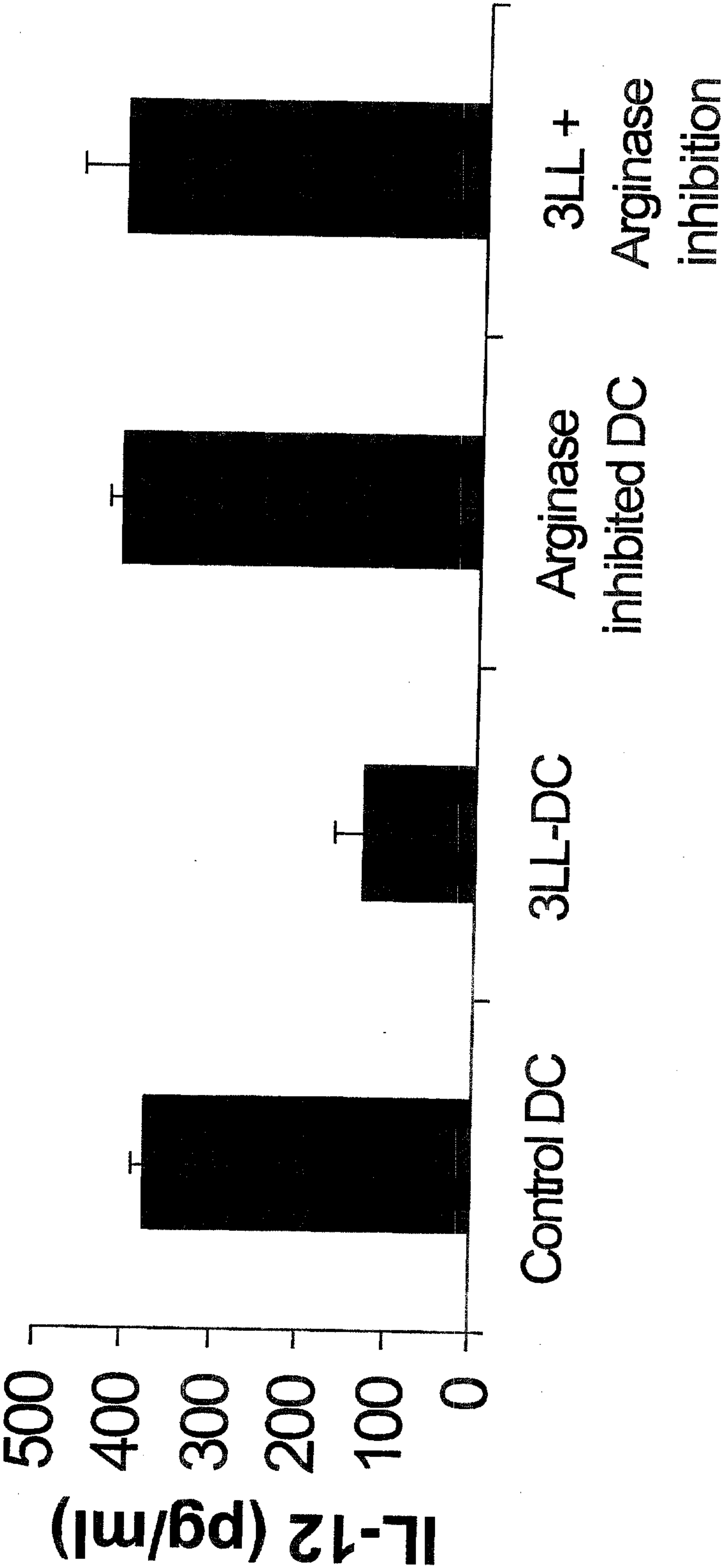


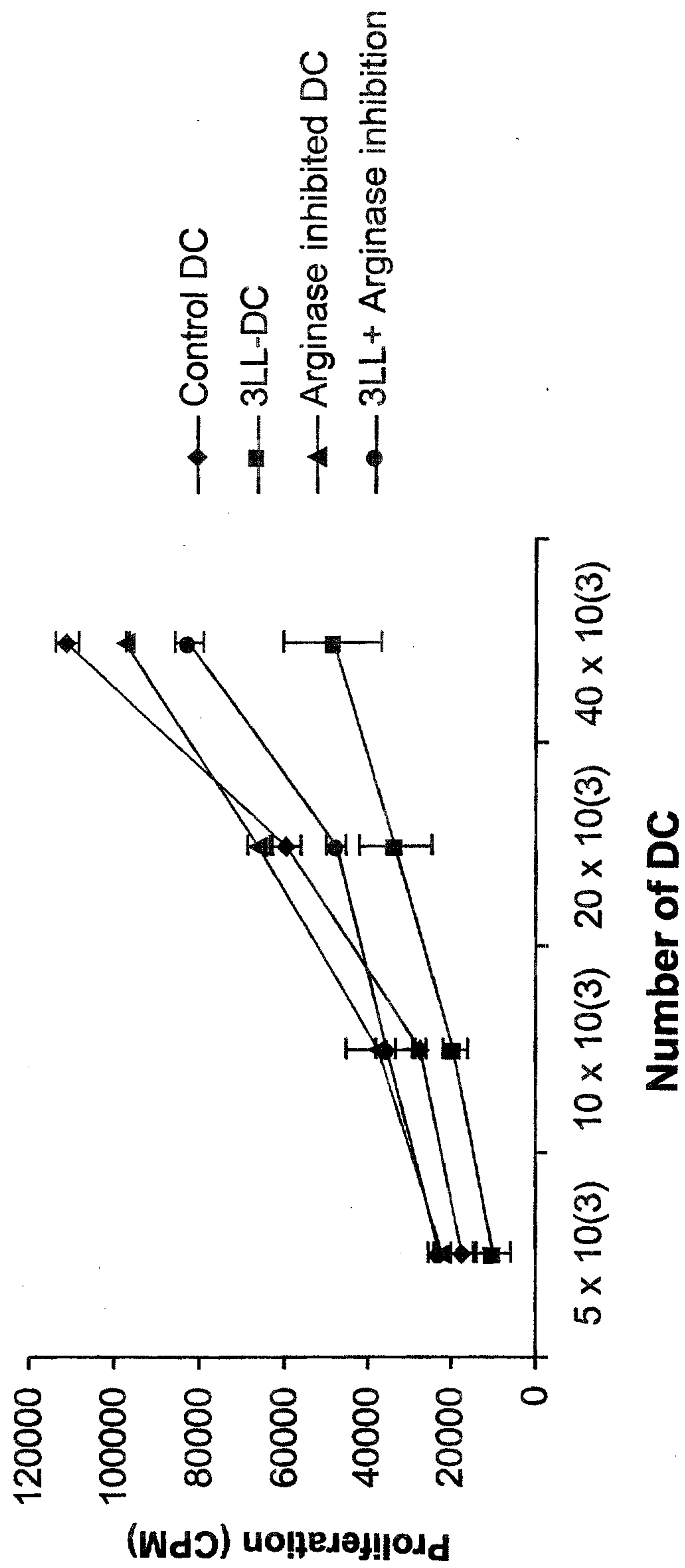
Figure 4

Figure 5

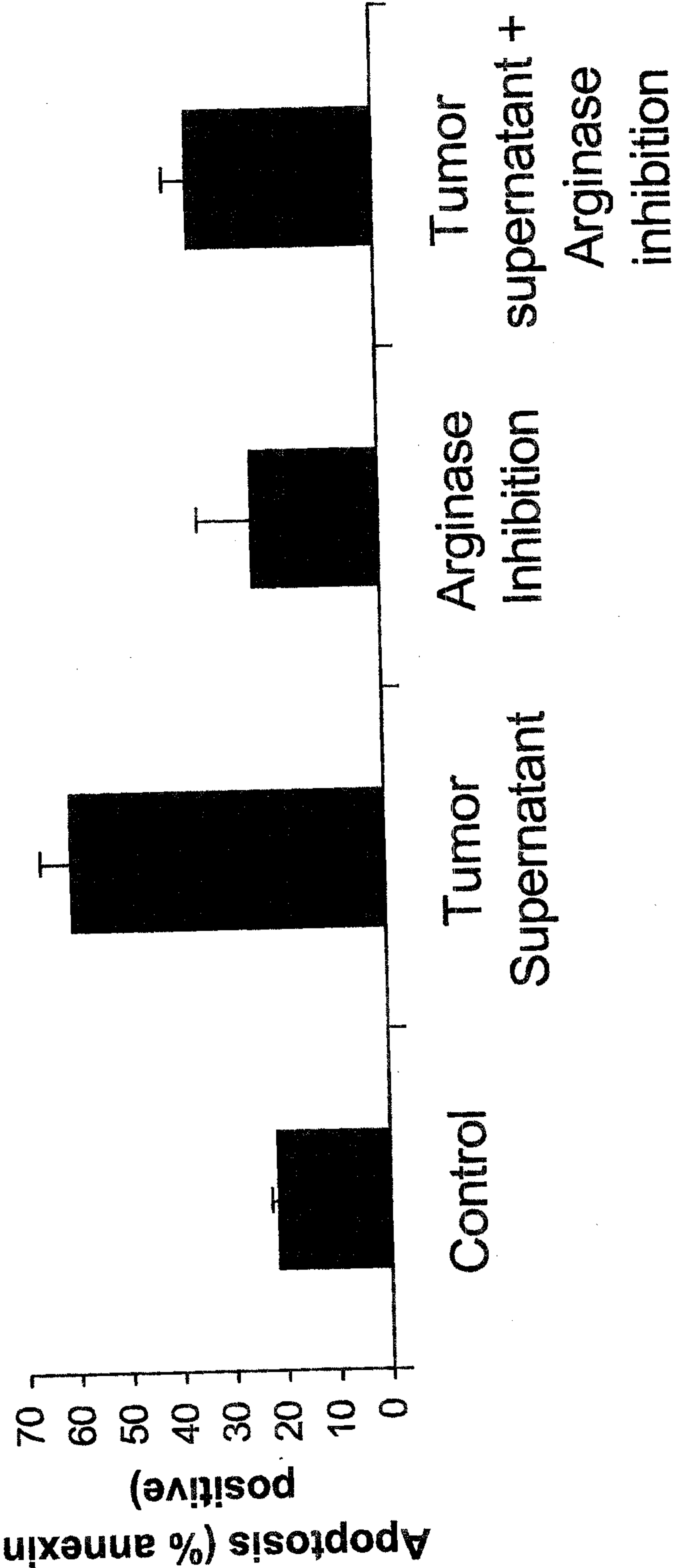


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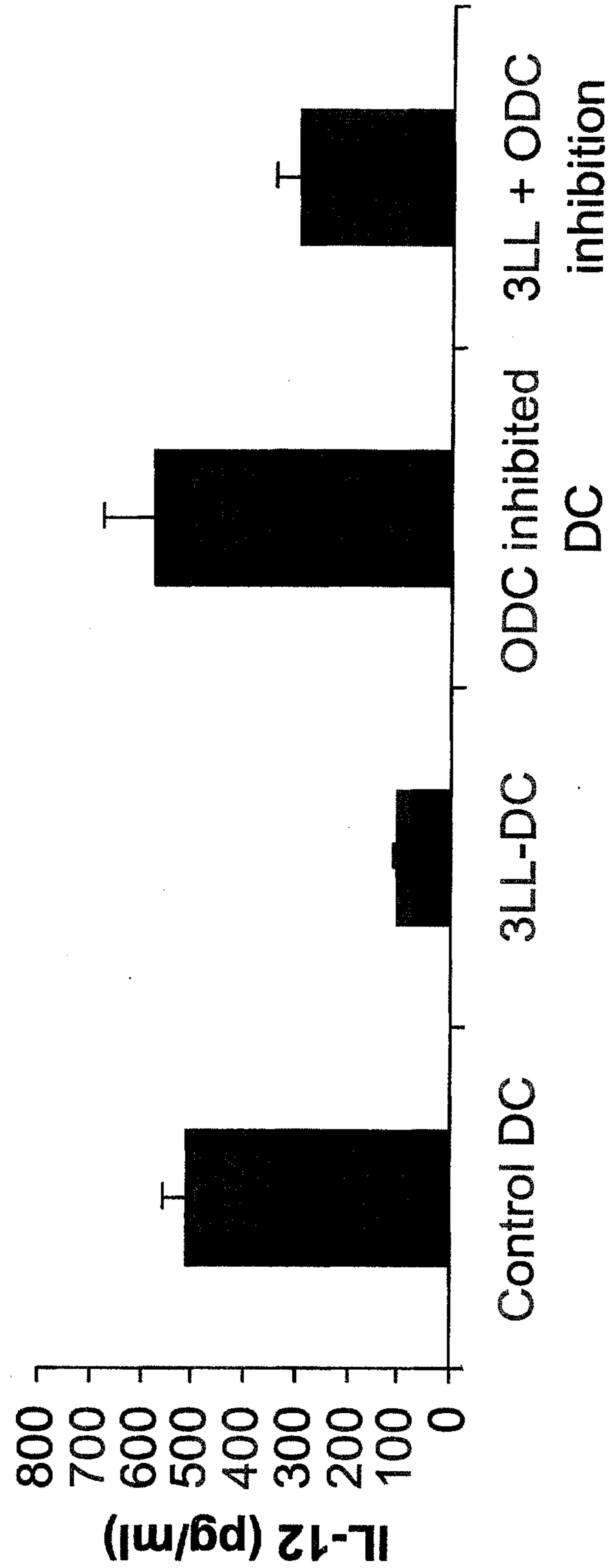


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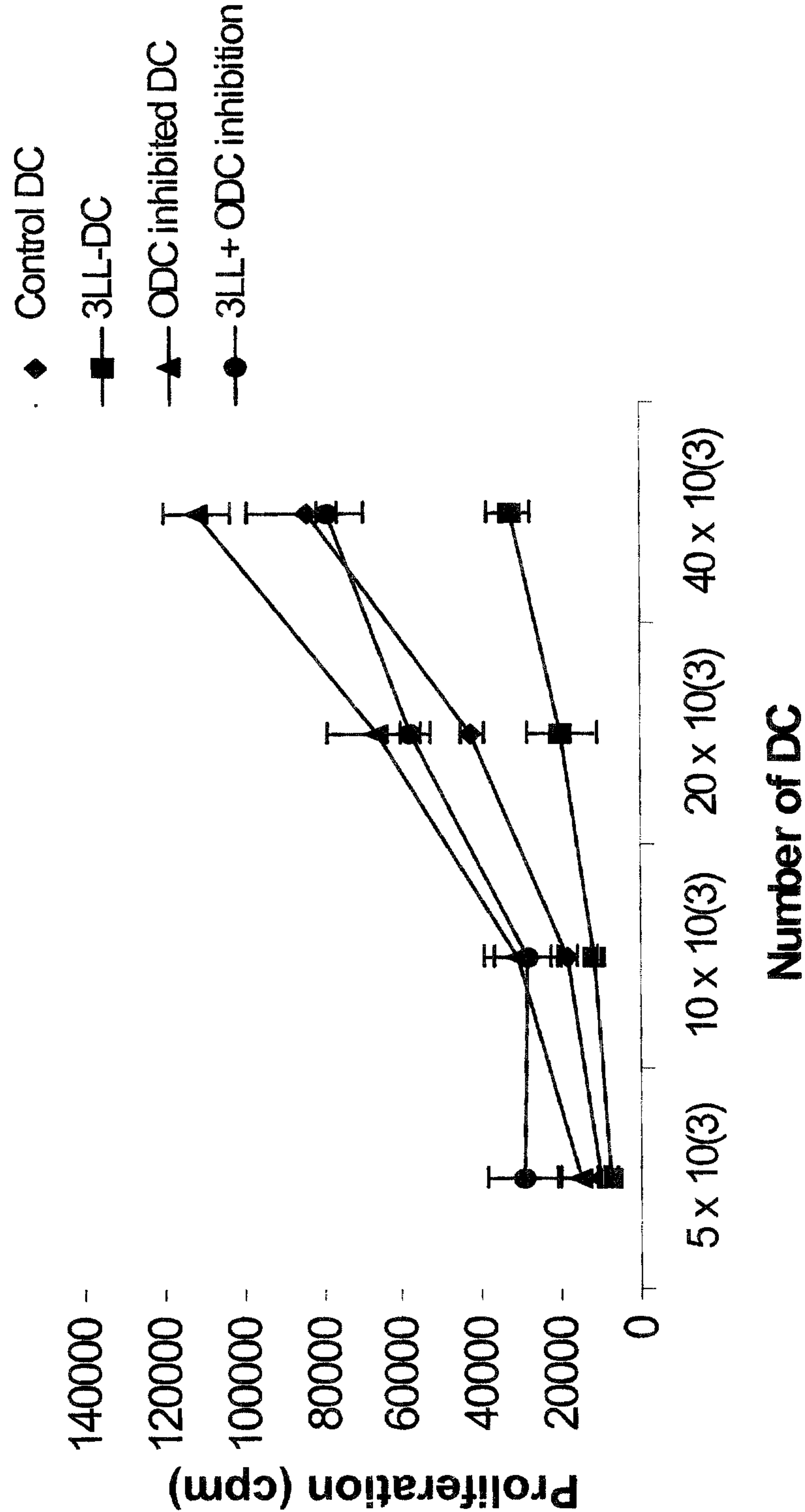


Figure 8

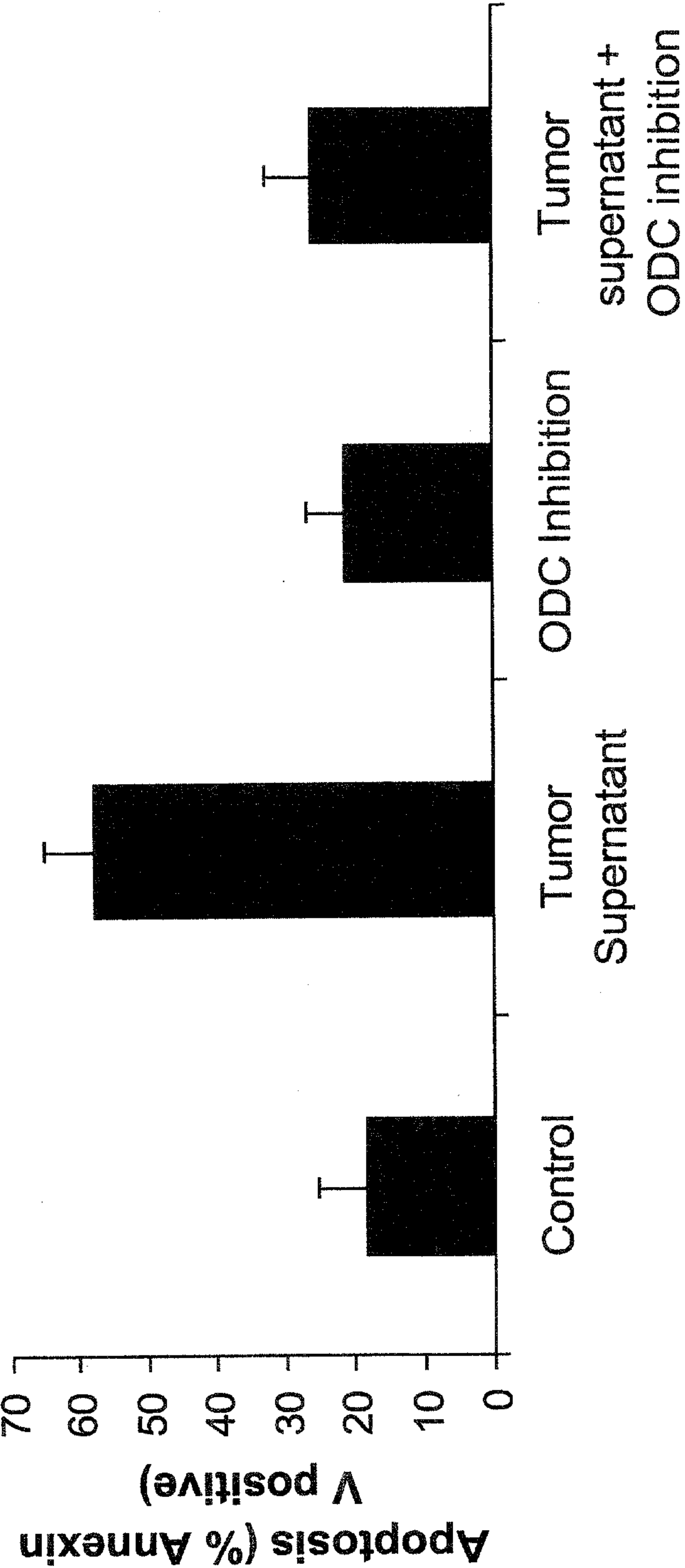


Figure 9

