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The effects of stress hormones on antibacterial and antiviral functions of resident peritoneal macrophages (MO) were determined. All studies were performed using a serum-free, chemically defined, medium, which eliminates complications which may arise as the result of hormone levels in fetal bovine serum. Restriction of herpes simplex virus type 1 (HSV-1) by resident peritoneal macrophages (MO) was essentially unaffected by the glucocorticoids, catecholamines, or isoproterenol. Phagocytosis of heat killed yeast Streptomyces cerevasiae was, however, inhibited by both dexamethose and corticosterone, but not by epinephrine or isoproterenol. The number of yeast phagocytosed per MO was inhibited by corticosterone in a dose dependent fashion. Killing of Listeria monocytogenes by resident peritoneal MO was not affected by the glucocorticoids. However, killing of L. monocytogenes by IFN or TNF/IFN activated MO (in vitro activation) appeared to be enhanced by dexamethasone.

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I. BACKGROUND

A. Anti-herpesvirus and anti-listeria activity of macrophages.

Mononuclear phagocytes (MP) are pivotal cells in specific immune responses and in natural resistance to viruses and bacteria (1). The MP system includes precursor pools in hemapoietic tissues, blood monocytes, as well as fixed and free tissue macrophages (MO) in the bone marrow, thymus, lymph nodes, spleen, liver, lung, intestine, and serosal cavities (e.g. peritoneal). The MP system also may include astrocytes of the nervous system, microglial cells of the central nervous system, osteoclasts in the bone, and dendritic cells in the lymphoreticular system.

Mims (2) and Gresser and Lang (3) first described a role for MP in resistance to virus infection, and these cells may exhibit both extrinsic and intrinsic resistance to viruses (4). Extrinsic resistance is defined as MP mediated inhibition of virus replication in other cells which are normally permissive for the virus (5,6). Intrinsic resistance is the inhibition of virus replication within the virus-infected MP (5,6).

The interaction between MP and herpesviruses is complex and varies with the source and type of MP. Murine resident peritoneal MO and activated MO are intrinsically resistant to infection with HSV-I, while elicited peritoneal MO are more susceptible to HSV-1 (7-10). Bone marrow derived murine MO are similar to elicited peritoneal MO in intrinsic resistance to HSV-1 (11,12). Human monocytes are intrinsically resistant to HSV-1 infections, but become less resistant after recruitment into the tissues during during inflammation (elicitation) in vivo, or during differentiation to MO in in vitro culture systems (13-17). Similarly, a human monocyte cell line U937 is intrinsically resistant to HSV-1, and differentiation of U937 with phobol esters decreases intrinsic resistance (18).

The mechanisms by which HSV-1 is restricted intrinsically by MP have not been completely defined, but also appear to vary with the type of MP. Considerable amounts of the immediate early (ICP-4) and delayed early (VP-5) HSV-specific protein are produced in murine resident peritoneal MO and activated peritoneal MO following infection. However, little viral DNA synthesis occurs and little to no infectious virus is produced (10,11). Elicted murine peritoneal MO are semi-permissive for HSV-1. Immediate early and early antigens are expressed and moderate levels of viral DNA and infectious virus are produced (11). In the undifferentiated U937 human monocyte cell line, restriction of the HSV-1 occurs earlier- prior to production of the immediate early protein ICP4 (19).

Control of or recovery from infections with Listeria monocytogenes also requires MO. As with restriction of HSV-1 infection, MO are heterogeneous in their response to Listeria. Inflammatory MO and MO from immune animals generally kill intracellular Listeria (20).

However, reports on anti-listeria activity of resident peritoneal MO are contradictory. Czuprynski et al.(21) report that resident murine peritoneal MO do not kill L. monocytogenes. Portnoy et al. (22,23), using an assay which can measure both bacterial killing and bacterial growth report that about 90% of Listeria organisms are killed within 2 hours following in vitro infection of freshly isolated peritoneal MO, but that the surviving bacteria persist. However, in those studies, if the MO were allowed to rest in vitro for 48 hours prior to infection, the bacteria would actually double in number three times within 8 hours of the initial killing. MO activation is important in resistance to Listeria. IFN and TNF, MO activation factors, enhance resistance to Listeria in vivo (24). Recently, Portnoy et al. (22) have demonstrated that treatment of resident murine peritoneal MO with a combination of rIFN v and rTNF promoted killing of intracellular L. monocytogenes. TNF alone had no effect, while IFN alone induced only partial resistance.

B. MO and neuroendocrine mediators of stress.

Stress often alters host resistance and immunity (24-26). Specific immunologic/host resistance sequelae to stress are dependent upon a variety of factors: the type /duration of stress (27); the type of immune response or infectious agent measured; and age, sex, genetic background, and general health status of the host (27). It is generally believed that the biologic sequelae to stress are mediated, either directly or indirectly, by neuroendocrine changes that occur in response to stressors.

Major neuroendocrine mediators of the peripheral stress response include products released from the anterior pituitary, adrenal glands and peripheral sympathetic nerves. For example, adrenocorticotropin (ACTH), B endorphin, prolactin, and growth hormone are released from the anterior pituitary. Glucorticoids are synthesized and released from the adrenal cortex. Met-enkephalin and catecholamines are released from the adrenal medulla, and norepinephrine is released from the peripheral nerve terminals (27-30).

MP exercise extrinsic and intrinsic antimicrobial functions in vivo where the environmental milieu may contain, at any one time, these neuroendocrine mediators among others. This is of particular importance because MO have receptors for or can be modulated by several stress hormones/peptides, e.g. ACTH, prolactin, opiates, catecholamines and glucocorticoids (31-33).

The effects of these stress hormones/peptides on MO vary. In some instances, two hormones may exert apparently opposing actions on MO functions. For example, prolactin reportedly stimulated superoxide production in porcine peripheral blood monocytes (34); whereas, B endorphin 1-31 suppressed zymosan induced superoxide production by human monocytes (35). In other cases, reports on the effects of a single stress hormone on MO appear to contradict each other. For example, hydrocortisone had no effect on latex phagocytosis or antibody dependent phagocytosis by MO cell lines, but dexamethasone and hydrocortisone

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inhibited phagocytosis of yeast by resident peritoneal MO (36,37). Met-enkephalin enhanced ADCC of murine peritoneal MO in one study, yet suppressed ADCC of the MO cell line RAW264 in another study (38,39).

The effects of stress hormones on MO activation by interferons (IFN) has received the most attention. ACTH, which reportedly is produced by lymphocytes in response to Newcastles disease virus (40), suppressed IFN activation of elicited murine peritoneal MO for antitumor activity (41). Norepinephrine and glucocorticoids also suppressed antitumor activity of INF activated MO (42,43). In contrast, leu-enkephalin augmented tumoricidal activity of murine elicited peritoneal MO activation by IFN α/β plus lipopolysaccharide (LPS) (44). Koff and Dunegan (41), however, reported that INF activation of elicited peritoneal MO for antitumor activity was unaffected by a-endorphin, B endorphin, leu-enkephalin, and met-enkephalin. Glucocorticoids inhibited IFN induced Ia antigen expression in a number of studies (42,44-46), and inhibited IL-1 production from immune/activated MO. However, glucocorticoids plus IFN induced expression of a new antigen on peripheral blood monocytes (47). Other studies have examined the effects of stress hormones on MO specific growth factors. For example, several studies have reported that glucocorticoids inhibit or reduce colony stimulating factor induced proliferation and differentiation (colony formation) of MO precursors (48-50).

Few studies have described the effects of stress hormones on antiviral functions of MO. Koff and Dunegan (51) compared the effects of several stress hormones/peptides on MO extrinsic antiviral activity. They reported that both norepinephrine and epinephrine blocked activation of MO by INF for extrinsic resistance to HSV; whereas, ACTH and B endorphin had no effect. We have observed that several of the glucocorticoids, epinephrine, norepinephrine and isoproterenol do not decrease the intrinsic antiherpesvirus activity of resident murine peritoneal MO, as measured by an increase in virus production or persistance at 72 hours post infection. However, dexamethasone and corticosterone, in our system, increased intrinsic resistance of murine thioglycollate (TG) elicited peritoneal MO to HSV-1 (Tables 5,6--preliminary data); whereas, certain concentrations of isoproterenol appeared to decrease resistance.

No studies, to date, have determined the effects of stress hormones on resistance to Listeria infections. The fact that Listeria infections are more severe in pregnant women suggests, although in no way proves, that neuroendocrine influences may alter resistance. In addition, no studies have determined the effects of the stress hormones on cytokine induced alterations in anti-listeria activity of MO.

II. PROGRESS REPORT.

In the first year of this project, we compared several serum-free, chemically defined media for their ability to support MO in culture and in our assays. We selected HL-1, Ventrex Corp. as the optimal medium. All further experiments were carried out in HL-1 medium in order to

avoid complications of hormone concentrations in fetal bovine serum (52).

A. Effects of glucocorticoids on general MO parameters.

In year one, we established that dexamethasone and corticosterone had mimimal effects on MO viability and spreading in culture or on the ectoenzyme phenotype (markers of MO activation) of resident peritoneal MO.

To determine whether our serum-free culture system would permit detection of glucocorticoid modulation of MO, we repeated and extended published observations of Grasso et. al (37) who demonstrated that glucocorticoids (dexamethasone and hydrocortisone) inhibited phagocytosis of heat-killed yeast by murine resident peritoneal MO. For these studies, resident peritoneal MO were cultured on 6 chamber culture slides for 24-48 hours in the presence or absence of various concentrations of dexamethasone or corticosterone. Heat-killed S. cerevisiae were added to the cultures for 1 hour. MO were vigorously washed to removed attached but non-phagocytosed yeast particles. The coverslips or slides were stained with Wright's giemsa, and the number of MO which had ingested yeast particles was detemined.

Our results were similar to those reported by Grasso et al.(37) for dexamethasone, which suppressed phagocytosis of yeast in resident peritoneal MO. We extended the experiments to include the murine endogenous glucocorticoid, corticosterone, which also suppressed phagocytosis of yeast. At all time points measured, 10^{-6} M corticosterone and dexamethasone treatment resulted in fewer phagocytic MO (Figures 1,2).

The number of yeast particles engulfed/ phagocytic MO was determined at 30 minutes, when resident control MO exhibited maximal phagocytosis. A clear dose response effect was observed with corticosterone (Figure 3). MO which had been treated with 10^{-6} M corticosterone averaged 3.5 yeast particles per phagocytic cell, and those treated 10^{-10} M exhibited a phagocytic index comparable to the controls.

The effects of epinephrine and isoproteronol on phagocytosis were also determined. Neither epinephrine nor isoproteronol had any effect on the rate or magnitude of phagocytosis of heat killed yeast by resident MO (data not shown).

B. Effects of stress hormones on antiviral activity.

In year 1, we determined that neither dexamethasone nor methylprednisilone had any effect on restriction of HSV-1 by resident peritoneal MO as measured by recoverable infectious virus. In year 2, we have established that neither corticosterone (Figure 4), epinephrine, isoproterenol, norepinephrine nor adrenocorticotropin hormone (ACTH) (data not shown) altered HSV-1 restriction by resident peritoneal MO.

C. Effect of stress hormones on anti-listeria properties of MO.

The effect of dexamethasone on the ability of resident peritoneal MO to kill L. monocytogenes was determined. For these studies, 1-2 X 10^7 resident peritoneal MO were isolated and cultured for 48 hours on 12mm circular glass coverslips in a 35mm dish. The cultures were infected with viable L. monocytogenes (approx. 1 organism/10 MO) for 30 minutes. Gentamycin was added after 30 minutes to kill non-engulfed organisms. Intracellular organisms were determined by placing one coverslip in sterile water, which lysed the MO and released intracellular Listeria. Aliquots of the lystate were plated onto brain heart infusion agar plates, and the number of colonies was counted. In three separate experiments (Figure 5), dexamethasone had no effect on the ability of MO to kill L. monocytogenes. MO can be activated in vitro for increased anti-listeria activity by IFN or by a combination of TNF/IFN.

MO which had been activated in culture with gamma interferon (r IFN-100U/ml) were more efficient in killing Listeria than resident MO. However, in two separate experiments,listericidal activity of activated MO was partially enhanced by the glucocorticoids. (Figure 6). Although, effects observed at 10^{-8} M and 10^{-10}M concentrations were not much different than the IFN control, a clear dose effect was demonstrable.

TNF/IFN act synergistically to activate MO for greater anti-listeria activity than IFN activated MO. Dexamethasone (10^{-6}M) enhanced the killing of listeria by TNF/IFN activated MO. We are currently investigating the underlying mechanism of these observations.

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IV. FIGURE LEGENDS.

Figure 1. Effect of dexamethasone (10^{-6}M) on the rate of phagocytosis of yeast. Data presented are from one representative experiment.

Figure 2. Effect of corticosterone (10^{-6}M) on the rate of phagocytosis of yeast. Data presented are from one representative experiment.

Figure 3. A dose reponse effect of corticosterone on the number of yeast particles phagocytized per MO. Corticosterone concentrations ranged from 10^{-4}M to 10^{-9} M.

Figure 4. Effect of corticosterone on restriction of HSV-1 by resident peritoneal MO. Data are presented as the average from two separate experiments. The data are expressed as $\log_{10}$ plaque forming units of HSV-1 per 10^6 MO. Vero cells represent a permissive virus production.

Figure 5. Effect of Dexamethasone on Anti-listeria activity of resident peritoneal MO. Data presented are from one representative experiment. Three experiments were done.

Figure 6. Effect of dexamethasone on activation of MO by IFN (100 U/ml) for increased anti-listeria activity. Data represent one of two simlar experiments.

Figure 7. Effect of dexamethasone on activation of MO by TNF (1000 U)/IFN (100 U) for increase anti-listeria activity. Data represent one of two similar experiments.

FIGURE 1

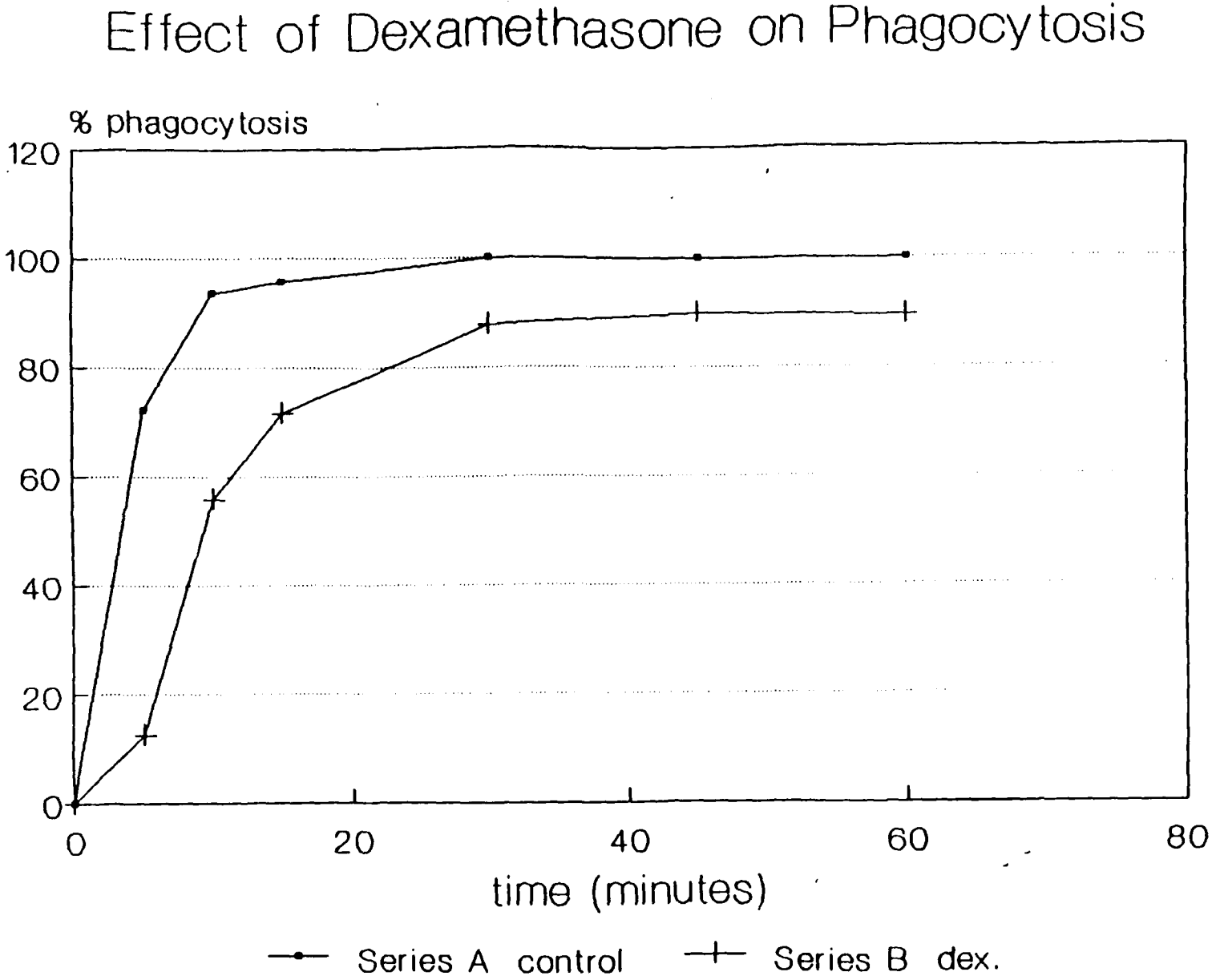

Effect of Dexamethasone on Phagocytosis
% phagocytosis
120
100
80
60
40
20
0
0
20
40
60
80
time (minutes)
Series A control
Series B dex.

FIGURE 2

Effect of Corticosterone on Phagocytosis

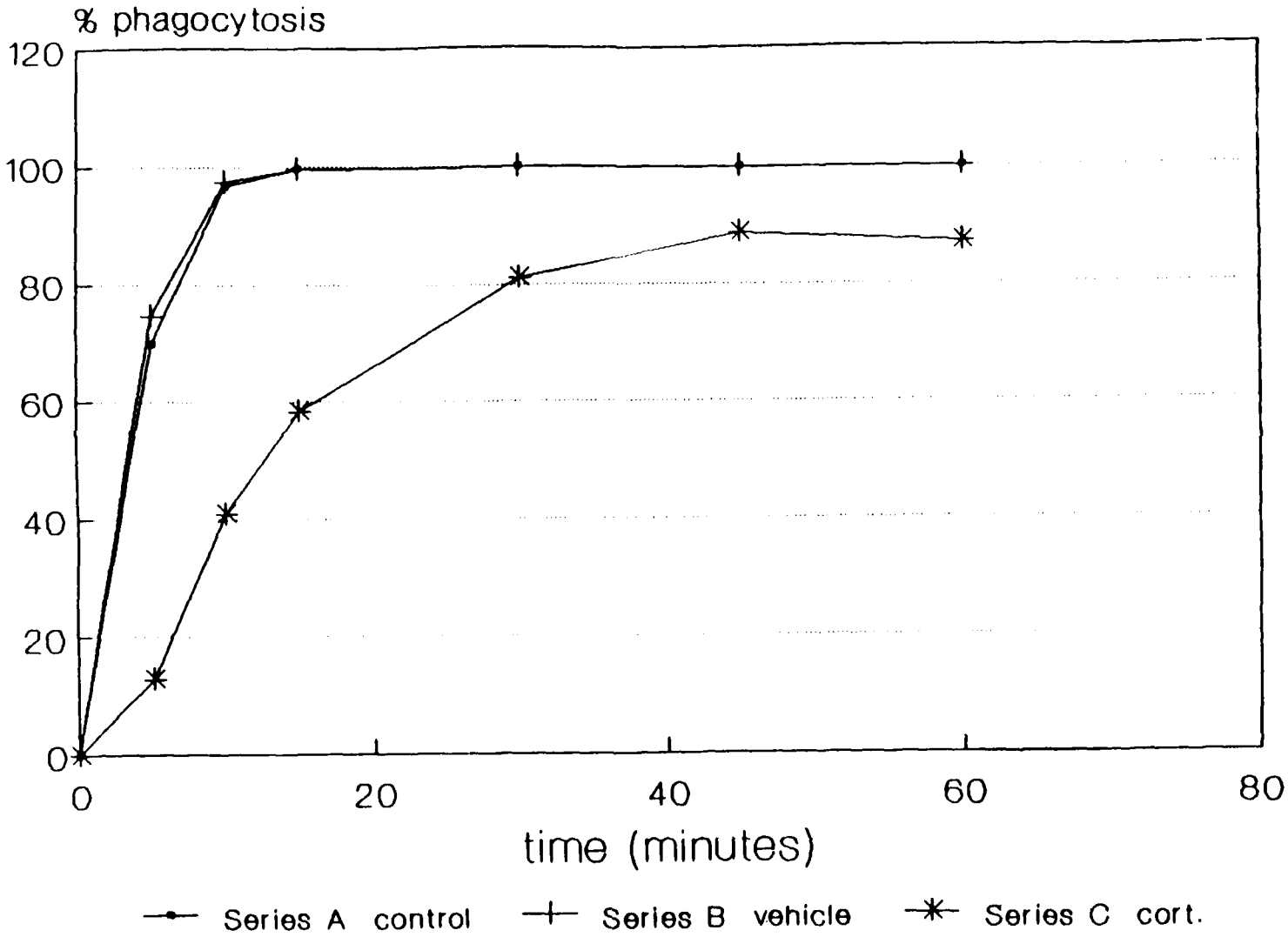


FIGURE 3

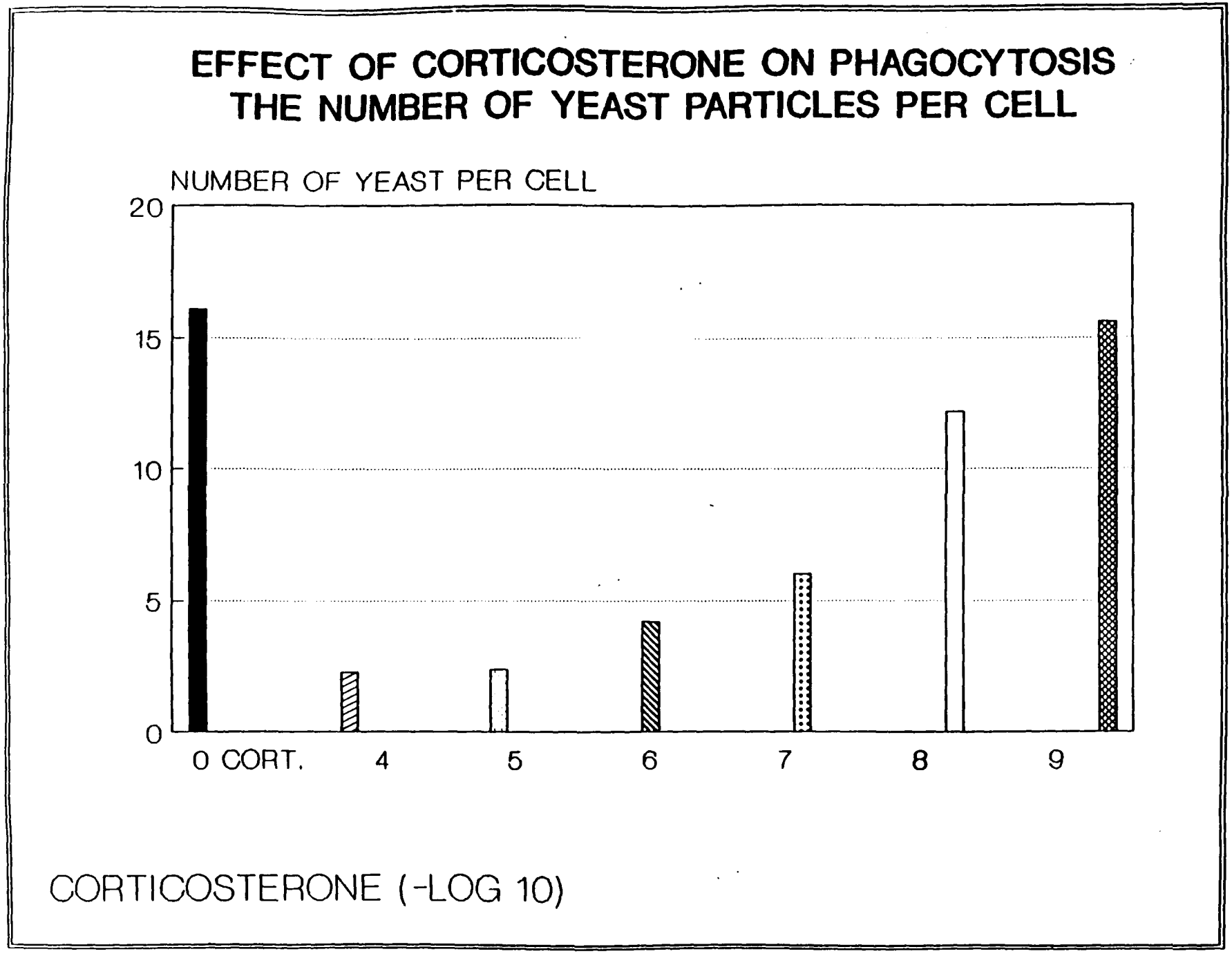

EFFECT OF CORTICOSTERONE ON PHAGOCYTOSIS
THE NUMBER OF YEAST PARTICLES PER CELL
NUMBER OF YEAST PER CELL
20
15
10
5
0
0 CORT.
4
5
6
7
8
9
CORTICOSTERONE (-LOG 10)

FIGURE 4

EFFECT OF CORTICOSTERONE ON RESTRICTION OF HSV-1 BY RESIDENT PERITONEAL MACROPHAGES

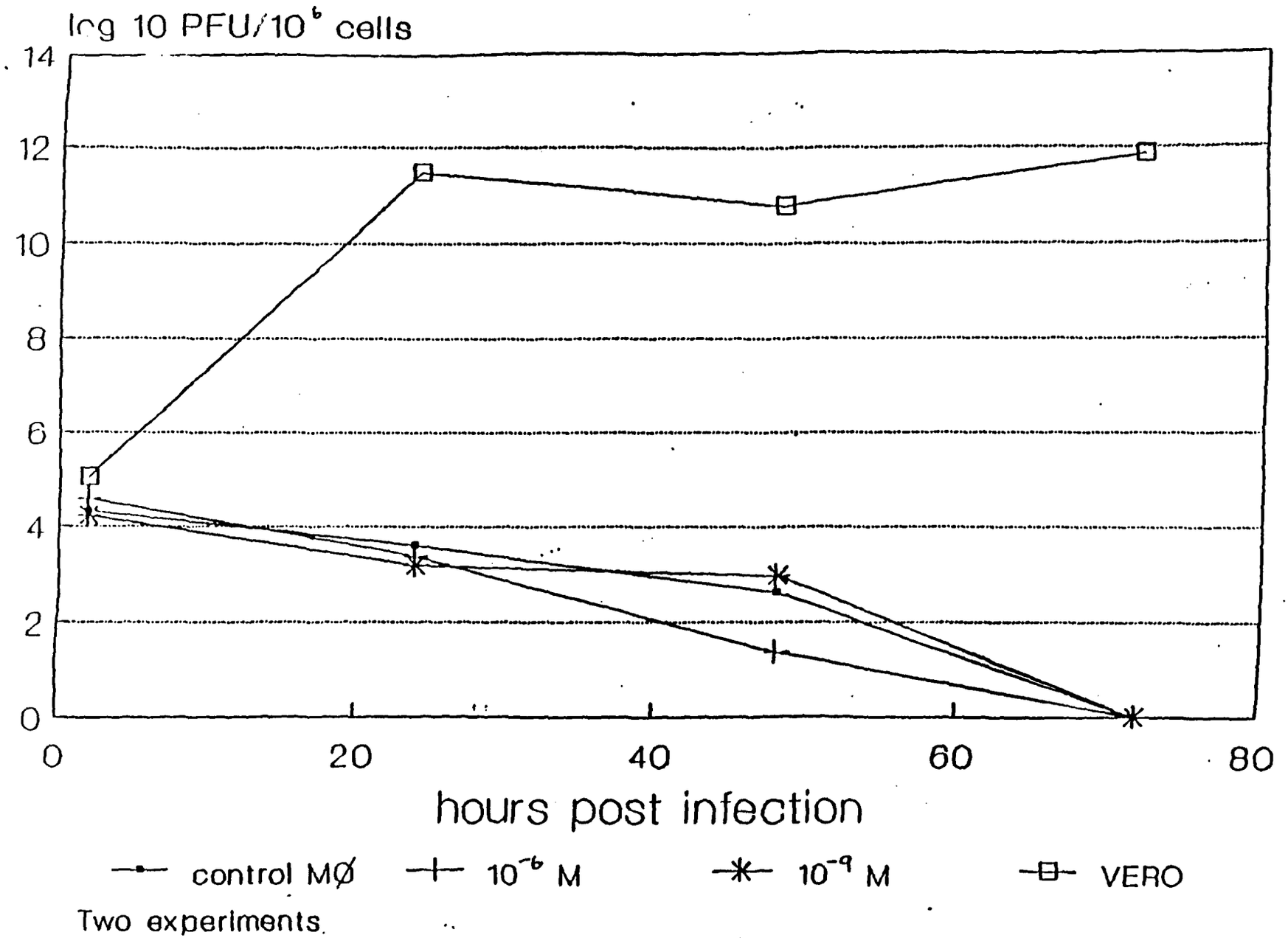


FIGURE 5

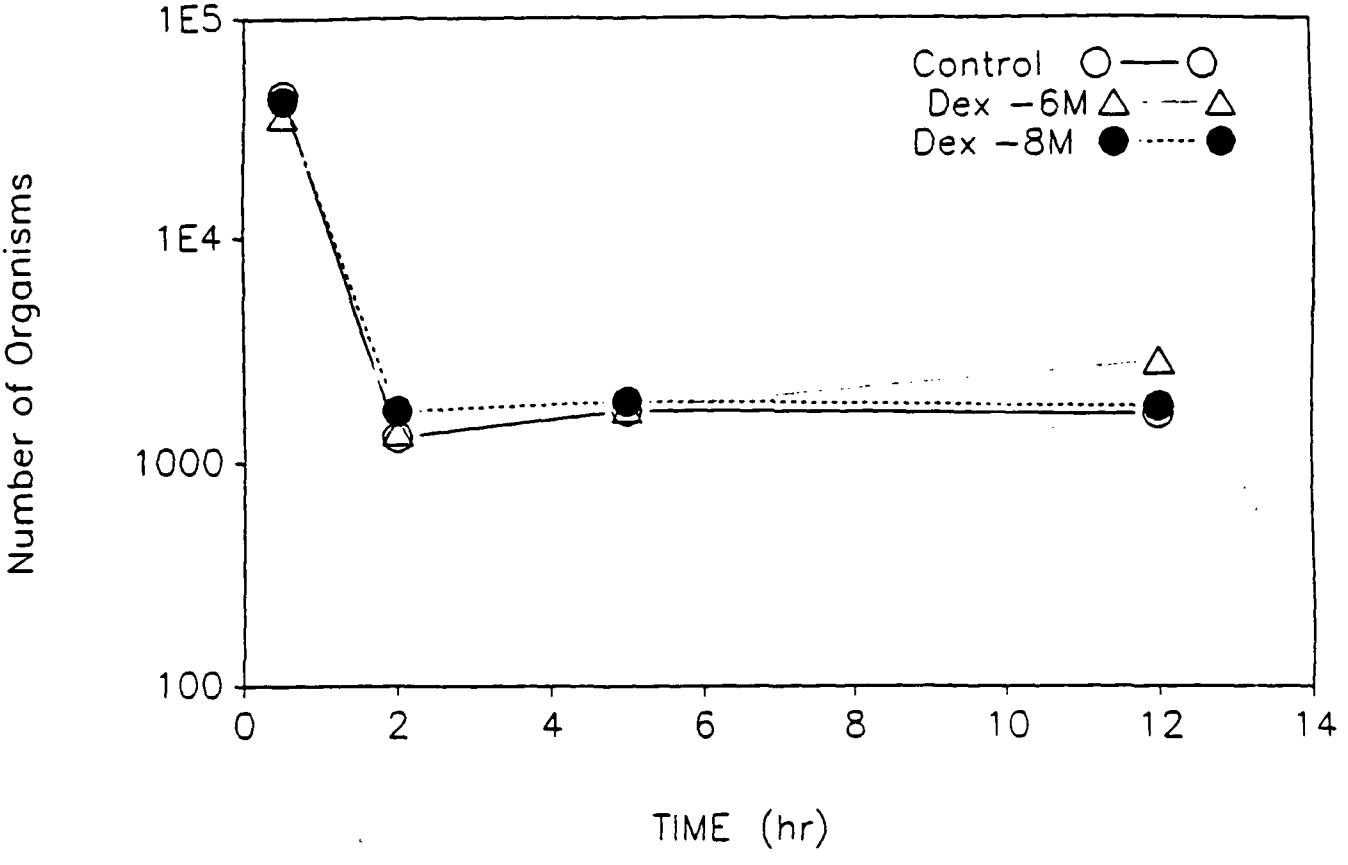

1E5
1E4
1000
100
0
2
4
6
8
10
12
14
Control
Dex -6M
Dex -8M
Number of Organisms
TIME (hr)

FIGURE 6

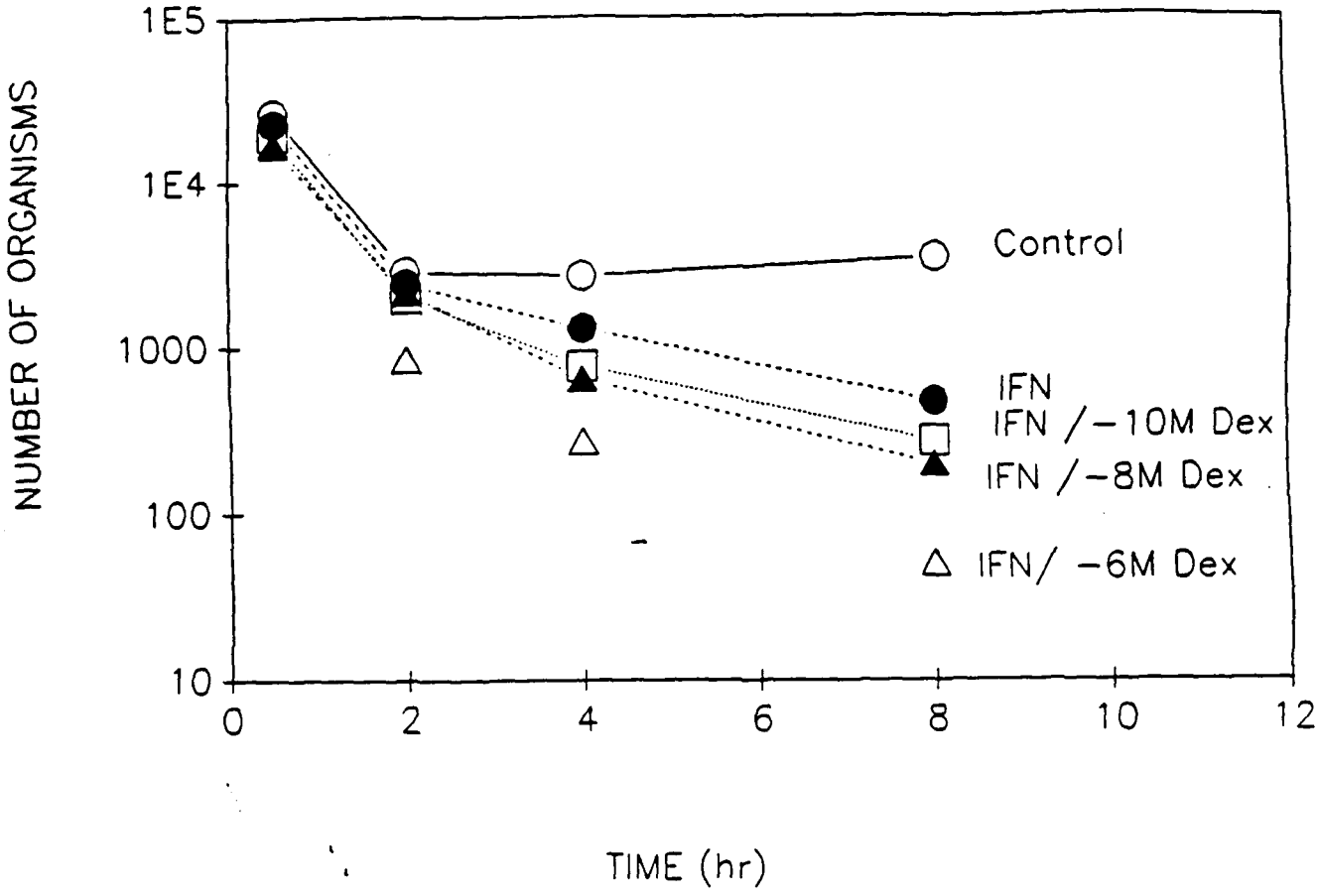
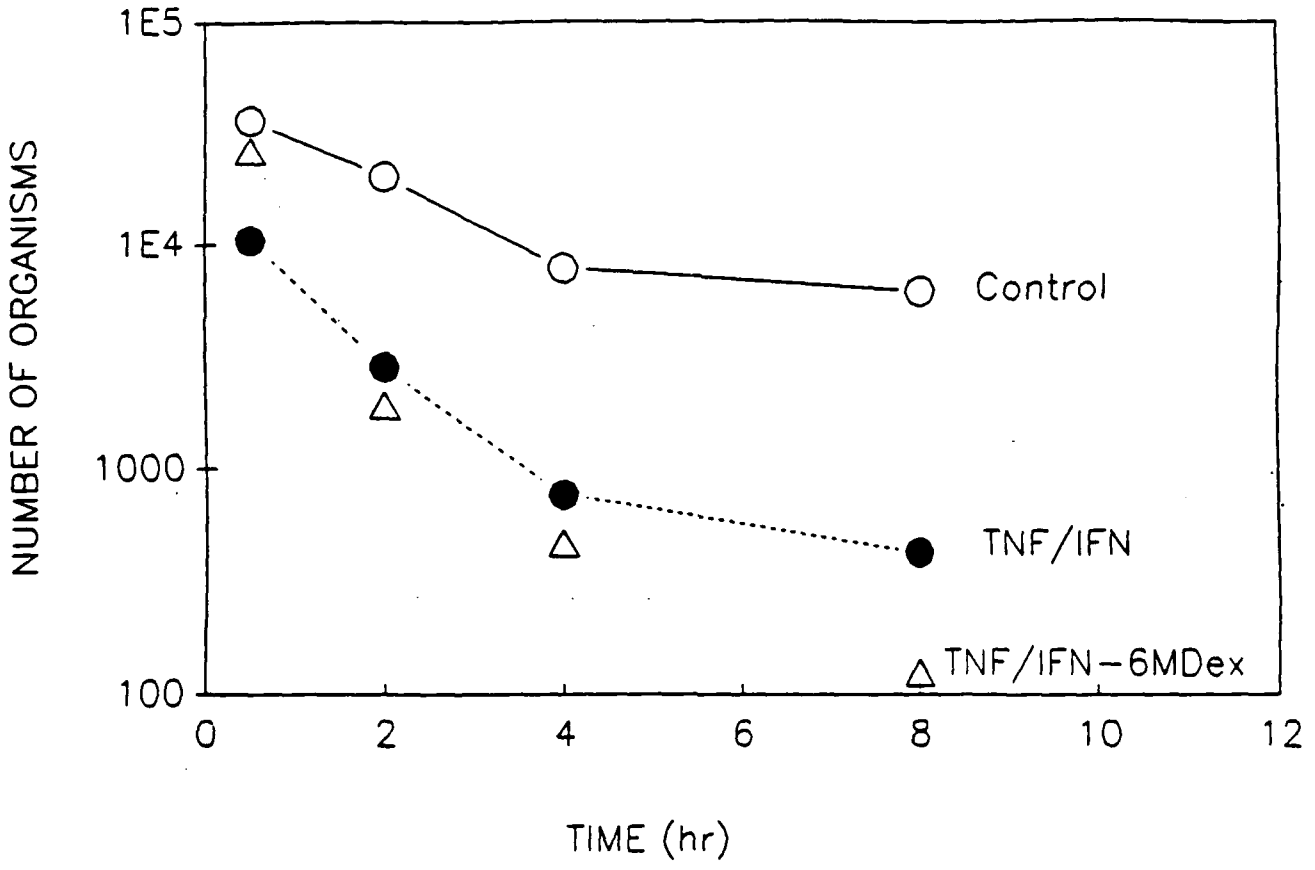

1E5
1E4
1000
100
10
NUMBER OF ORGANISMS
0
2
4
6
8
10
12
TIME (hr)
Control
IFN
IFN /−10M Dex
IFN /−8M Dex
IFN/ −6M Dex

FIGURE 7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NUMBER OF ORGANISMS
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2
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TIME (hr)
Control
TNF/IFN
TNF/IFN-6MDex