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CYTOKINES AND IMMUNE SURVEILLANCE IN HUMANS

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INTRODUCTION

Evidence from both human and rodent studies has indicated that alterations in immunological parameters occur after space flight [1,2]. Among the parameters shown, by us and others, to be affected is the production of interferons [1-3]. Interferons are a family of cytokines that are antiviral and play a major role in regulating immune responses that control resistance to infection [1-3]. Alterations in interferon and other cytokine production and activity could result in changes in immunity and a possible compromise of host defenses against both opportunistic and external infections.

The purpose of the present study is to explore further the effects of space flight on cyotokines and cytokine-directed immunological function.

METHODS

Among the tests carried out are interferon-alpha production, interferon-gamma production, interleukin-1 and -2 production, signal transduction in neutrophils, signal transduction in monocytes, and monocyte phagocytic activity. The experiments will be performed using peripheral blood obtained from human subjects.

In it our intent to eventually carry out these experiments using astronauts as subjects to determine the effects of space flight on cytokine production and activity. However. these subjects are not currently available. Until they become available, we will carry out these experiments using subjects maintained in the bed-rest model for microgravity.

RESULTS AND DISCUSSION

In the past six months we have continued our analysis of the bed-rest subjects. We have coordinated our findings with that of the Faculte de Medicine at Toulouse, France and have come up with the following results. We have noted that interleukin-1 not only has osteoclast activating activity, but can participate in muscle wasting often observed after cachexia. This further highlights the significance of the increase in interleukin-1 activity after bed-rest in which changes in bone and muscle were observed. Additional coordination has led to the development of a joint manuscript involving immunological results of both French and US Bed-rest studies. The coordination will allow a direct contrast of the US and French bed-rest techniques. This manuscript is in preparation.

We are currently developing additional combined bed-rest and isolation studies with the French. We will repeat and expand our findings. This should lead to information that could aid in designing future space flight studies.

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PUBLICATIONS

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THE ROLE OF CYTOKINES IN IMMUNE CHANGES INDUCED BY SPACE FLIGHT

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ABSTRACT

Over the years, it has become apparent that space flight alters many immune responses. Among the regulatory components of the immune response that has been shown to be affected by space flight is the cytokine network. Space flight, as well as model systems of space flight, have been shown to affect the production and action of various cytokines including interferons, interleukins, colony stimulating factors, and tumor necrosis factors. These changes have been shown not to involve a general shut-down of the cytokine network, but, rather, selective alterations of specific cytokine functions by space flight. The full breadth of changes in cytokines induced by space flight, as well as mechanisms, duration, adaptation, reversibility, and significance to resistance to infection and neoplastic diseases remain to be established.

THE ROLE OF CYTOKINES IN IMMUNE REGULATION

In recent years, it has become clear that cytokines play a significant role in immune regulation (1-3). A wide variety of cytokines, including interferons and interleukins, now appear to play major roles as signals among different cell populations and within cell populations. These cytokine signals can include positive-negative feedback interactions, such as the interaction between interleukin-10 and interferon-γ in regulation of proposed T-helper cell-1 (TH1) and T-helper cell-2 (TH2) activity (4).

The role of cytokines in regulating immune responses is complex and is under current extensive study throughout the world. It appears that the nature of the interactions of cytokines becomes more involved with every new discovery, and still undiscovered cytokines probably will take their appropriate place in immunoregulatory pathways.

Since it is now clear that space flight influences immunological parameters (5), it is imperative that the effects of space flight on the production and action of cytokines be explored. This type of study could yield extensive information on the mechanism of the effects of space flight on immune responses, as well as new information on how the immune response system interacts with other systems that are affected by space flight. This could give us information that would be useful for developing countermeasures to prevent or correct space flight-induced changes that are detrimental to immune responses. Moreover, information could be gained to provide insight into the fundamental mechanisms of how the immune system functions.

Space flight could influence cytokines at two fundamental levels. The first could involve recognition of the stimulus for cytokine production/action by the cells involved (Figure 1). This could involve such factors as cell-to-cell interaction, production and/or expression of extracellular matrix and/or receptor proteins and alterations in aggregation or kinetics of

receptor-stimulus interactions. The second could involve the effector phase of cytokine production/action (Figure 2). This could involve altered signal transduction patterns, altered transcription/translation levels, altered post-translational modification, and altered levels of secretion of final products.

For these reasons, studies have been initiated that use both modeling and actual in-flight experiments to determine how cytokine production and action is affected during space flight. The main focus of this paper will be to review these studies.

INTERACTION OF CYTOKINES WITH OTHER REGULATORY SYSTEMS AFFECTED BY SPACE FLIGHT

Space flight produces broad effects on living systems (6). A description of many of these effects is contained in an issue of **Science** magazine that was dedicated totally to a description of the interactions of space flight with living systems (6). There are many factors involved in space flight, including the removal of the host from the normal gravitational field to a condition of microgravity (the near-zero gravity environment obtained in space craft during earth orbit) and exposure of the host to new forms of radiation and stress (6).

The immune system is not an isolated entity that acts solely in an independent fashion. The immune system interacts with other organs and tissue throughout the body. This interdependence among body systems leads to networks that can be very much affected by space flight whether or not every component of the network is affected directly by micorgravity. Therefore, effects of space flight on other parts of the body can have profound consequences for the immune system and cytokine production and function.

Among the bodily functions that interacts with the immune system and the cytokine network is the neuroendocrine system (7). This set of responses, so much affected by the

stress response of the host, interacts in many fashions with the immune system. Included in this interaction are the adrenal hormones such as corticosteroids, which can drastically alter many different immune responses (7). In addition to neuroendocrine interactions, the β-adrenergic system can also have major impact on the immune system in conveying the effects of stress (8).

It is clear, also, that musculoskeletal responses are affected profoundly by space flight (9). These responses can also influence the immune system. The potential interactions include the cytokine interleukin-1 having both immunological as well as muscle atrophy-inducing and osteoclast activating activities (10). In addition, 1,25-dihydroxyvitamin-D_3, that plays a major role in calcium and drug metabolism, also is produced by macrophages and can regulate their function (11).

In an attempt to differentiate between direct effects of microgravity and effects of other signals or factors on cytokines, both modeling on the ground and the use of cell cultures in flight experiments have been carried out. The results of these modelling and cell culture experiments have great value in predicting what may happen in space as well as in allowing the study of potential mechanisms. However, the results of these modelling and cell culture experiments may prove to differ from flight experiments with in tact hosts. For the modeling experiments, other factors in flight such as radiation and stress, may not be present. For the cell culture experiments, the immune cells are out of their normal "milieu", not permitting interactions with other body systems.

Therefore, the immune response in general, and cytokines in particular, may be very much influenced by changes in these as well as other, yet undefined, networks throughout the body. In addition to considering the effects of microgravity in space flight on the immune response, the effects of alterations in other systems must also be considered in planning and evaluating experiments. It is very difficult to differentiate between effects of microgravity and

effects of other systems on immune responses. For this reason, throughout this review we will refer only to "effects of space flight on immunity" rather than using the term "effects of microgravity on immunity".

In this review, we will attempt to cover all types of space-related experiments involving cytokines that have been carried out. However, the experiments must be interpreted bearing in mind potential interactions of the immune systems with other body functions.

EFFECTS OF GROUND BASED MODELS ON CYTOKINE PRODUCTION AND ACTION

Several models for microgravity and other space flight factors have been used on the ground to study components of the cytokine system, and to plan and prepare for the limited opportunities for actual space flight experiments. Among these are a tissue culture system for keeping cells from interacting properly, an isolation system, and antiorthostatic, hypokinetic, hypodynamic suspension of rodents (Table 1).

The cell culture system was developed by Gmünder and associates (12). In this system, plastic tissue culture flasks and dishes were coated with different thickness of poly-HEMA films. Poly-HEMA created a film on the plastic that decreased cell spreading and interaction as the thickness of the film was increased. This would be similar to changes in interaction of cells expected to be induced by space flight. Purified peripheral blood mononuclear cells from healthy human donors were placed in culture in the poly-HEMA-coated dishes. As the concentration of poly-HEMA increased, the cell adhesiveness and interaction decreased, and this correlated with decreased production of interferon-γ when the cultures were challenged with a mitogen (12). These data suggest that cytokine production could be altered by space flight due to changes in interaction and adhesiveness of cells induced by the flight.

In the isolation system, a single, healthy 27 year-old female was isolated for 5 months

in a cavern. This individual had no concept of diurnal variation and no contact with the outside world except via computer terminal communication (13). This type of isolation is similar to that which could occur for astronauts or cosmonauts during long-term space flight or during colonization of a planet. When peripheral blood leukocytes of this individual were stimulated with mitogens to induce interferon, the level of production of interferon-γ was elevated throughout the isolation period compared to a normal population. The level of interferon production returned to normal immediately after the completion of isolation (13). The increase in interferon-γ also correlated with an increase in natural killer cell activity. These data suggest that the isolation component of space flight alone can affect cytokine production; however, the interpretation of these data must take into account the following caveats: 1) there was only one individual tested in this case, and 2) the individual was on a calcium-depleted diet, that could have affected the results observed.

The antiorthostatic, hypokinetic, hypodynamic model has been used for several years to model the effects of microgravity on biological systems (14-16). The effects of suspension on immune response has been reviewed elsewhere in detail in this volume (17), so the effects on cytokines will only be reviewed briefly here. Both tail and harness suspension have been used to study the effects of suspension on cytokine production (18-20). Experiments were carried out on both rats and mice, and results were similar in all cases. When whole animals were challenged with an interferon-α/β inducer immediately after suspension, the production of the interferon was reduced significantly (18,19). When the animals were allowed to recover from suspension prior to challenge or when orthostatically suspended animals were used (controls for the stress of suspension without a head-down tilt), there was no decrease in interferon production. When mice were challenged with encepahlomyocarditis-D virus, a virus to which the mice were normally 100% resistant, the majority of antiorthostatically suspended mice showed pathological indications of successful infection. Control, orthostatically suspended mice

could not be infected (20). Susceptibility to pathological effects of virus infection correlated with decreased interferon production. These results suggest that the antiorthostatic suspension system, that models some effects of space flight, could result in altered interferon production and increased susceptibility to infection.

An additional test was carried out by placing rats in harness-type antiorthostatic suspension (21). When spleen cells from these rats were removed and challenged with inducers, the following results were observed; no effect on interferon-α/β production, increased interferon-γ production, and decreased interleukins-1 and -2 production (21). Just placing the rats in harnesses restraint did not affect the interferon-γ response, but did decrease the interleukins-1 and 2 responses. These results suggest that *in vitro* challenge of spleen cells from suspended animals with inducers of cytokines can produce different results from *in vivo* challenge of suspended animals with inducer for production of interferon-α/β (18-21). In addition, it could be postulated that musculoskeletal unloading affected the interferon-γ response, but that the interleukin-1 and -2 responses were affected by the stress of restraint (21).

In one additional test, bone marrow cells from tail-suspended rats were tested for their ability to respond to stimulation with exogenous granulocyte/macrophage-colony stimulating factor (22). Their response was inhibited greatly, which was similar to the response seen in bone marrow cells from rats flown for two weeks in a parallel experiment aboard Cosmos 2044 (22).

From the above results, it appears that these on-ground test systems are useful for modeling the effects of space flight on cytokine production and action. They may not be so useful in modeling other aspects of immune responses.

EFFECTS OF SPACE FLIGHT ON CYTOKINE PRODUCTION AND ACTION

In Vitro Effects

There have been several studies of the effects of space flight on cultures of immunologically important cells (Table 2). It is important to note that cells in culture are out of their normal environment and are not subject to normal interaction with hormones, cytokines and other body systems. Therefore, results with cells in culture in space flight may differ from results with whole organisms in space flight.

In a combined Hungarian/Soviet study, peripheral blood leukocytes from Cosmonauts were placed in culture during a space flight and challenged with a variety of interferon-α/β inducers. The results of these studies indicated that interferon-α/β production was increased dramatically during flight (23-24). It is interesting to note that when blood was drawn from the same Cosmonauts upon return to earth and then the cells were challenged with inducers, the production of interferon-α/β was inhibited dramatically compared to production form cells of these same individuals obtained before flight(23-24). This was a clear indication that results with individual cells in culture can differ from results with the same cells when they are in the whole organism in the presence of other regulatory factors.

Limouse et al., (25) placed human T-lymphocyte and monocyte lines in culture in a Cosmos biosatellite flight. These cells, therefore, had the possibility of cell-to-cell interaction required for normal function and cytokine production. When these cells were challenged with monoclonal antibodies directed against the T-cell receptor complex, appropriate cell-to-cell interaction occurred and the monocytes produced interleukin-1 while the T-lymphocytes produced interleukin-2 at normal levels (25). However, when the cells were cultured

separately and challenged with inducers such as phorbol esters which could induce interleukins on the ground, the production of both interleukins-1 and -2 was inhibited severely (25). Therefore, space flight could affect the cellular target of phorbol esters, but did not have direct effects on cell metabolism that disrupted cell-to-cell interaction.

Chapes et al (26) flew separate cultures of bone marrow macrophages, murine spleen and lymph node cells, and human lymphocytes on Space Shuttle flights. When the bone marrow cells were stimulated with lipopolysaccharide, higher levels of interleukin -1 and tumor necrosis factor-α were produced than by ground controls. The same type of pattern was observed with murine spleen cells stimulated with polyriboinosinic-polyribocytidylic acid to produced interferon-α/β and murine and human lymphocytes stimulated with concanavalin-A to produce interferon-γ (26). These results are consistent with the Hungarian/Soviet results described above (23-24).

An additional cell culture study by Cogoli and associates (27) involved concanavalin-A stimulation of human peripheral blood leukocytes flown in cell culture aboard a short-term US Space Shuttle mission. Cells flown in suspension culture showed inhibition of activation, but cells attached to microcarrier beads showed enhanced production of interleukin-2, tumor necrosis factor-α, and interferon-γ. The authors postulated that attachment of the cells to the beads allowed for cell-to-cell interaction between macrophages and lymphocytes, resulting in enhanced production of cytokines. The suspended cells could not interact properly, yielding decreased interleukin-1 production and inhibiting the production of other cytokines. The authors feel that interleukin-1 was the crucial cytokine affected by space flight.

All of the tissue culture results indicate that there may be direct effects of microgravity on cells important to the production of cytokines. The effects of space flight on the whole organism may be different than that of individual cells because many different additional factors, such as stress hormones and other regulatory factors, may also come into play when the

immune cells are located in the intact host.

In Vivo Effects

All of the studies involving the effects of space flight on cytokines in whole organisms have involved the examination of cells from whole organisms immediately upon return of the whole organism to earth. Experiments have been carried out after both short-term (days to weeks) and long-term (months) space flight (Table 2). The performance of these studies requires living cells and the only way the studies can be performed completely in space is by using a fully equipped laboratory that will not be available until the "Space Station Freedom" or permanent manned moon or planetary colony are operational in the future. Although, for the purpose of this review, the studies are being called *in vivo*, it is appropriate to note that exposure to space flight conditions occurred to the whole organism but assays were carried out *in vitro* after return to the ground.

The first studies carried out in whole organisms in space are those mentioned previously about the effects of space flight on interferon-α production (23-24), When cells were obtained from Cosmonauts immediately after a short-term flight, interferon-α/β production was inhibited. Additional preliminary data indicate that alterations in cytokine production can occur in cells taken from astronauts after short-term space flight in the US Space Shuttle (28). Later studies by Konstantinova and her associates have found alterations in interferon-α, interferon-γ, and interleukin-2 production of cells obtained from Cosmonauts after long-term space flight (29). Although the results were variable and preliminary in nature, they did suggest that long-term space flight could have great impact on the cytokine network. Additional recent Franco/Soviet studies have suggested increases in interleukin-2 production but no

alteration in interleukin-1β production immediately after long-term flight of cosmonauts in the "Mir" space station (30). Again, space flight appears to be able to alter selective immunological parameters, but does not suppress the entire cytokine network.

Similar results were obtained when rats were flown on the SL-3 seven day US Space Shuttle mission. When spleen cells were challenged with mitogen upon return to earth, interferon-γ production was inhibited severely compared to ground controls (31). Interestingly, when the same culture supernatant fluids were examined for production of interleukin-3, production from the flight animals was normal compared to controls (31). This further substantiated the previous observations that space flight does not invoke a blanket shut down of the cytokine system, but rather, induces selective alterations in components of the cytokine pathway.

Additional studies on short-term space flight aboard the Cosmos 1887 and 2044 flights suggested that space flight could have great impact on the function of cytokines. Bone marrow cells from flown rats were treated with either purified natural macrophage colony stimulating factor (Cosmos 1887) or recombinant granulocyte/macrophage colony stimulating factor (Cosmos 2044) (22,32). In both cases, the ability to respond to the colony stimulating factors by producing colonies of cells was inhibited severely in cells from the flown rats compared to cells from control rats. Cells from the flown rats were inhibited more severely than cells from synchronous control rats (rats treated in the same fashion as the flight rats with the sole exception of flight), indicating that space flight conditions truly were responsible for the decreased response of the bone marrow cells (32).

Soviet investigators performed other studies during the Cosmos 1887 flight. They were able to show alterations in interferon-α/β, interferon-γ, interleukin-2, tumor necrosis factor-α, and osteoclast activating factor production by spleen cells after the 12 and 1/2 day space flight (29).

In an interesting recent study using lymph node cells from rats flown aboard the Cosmos 2044 mission, Nash and associates were able to show that interleukin-2 production by lymph node cells was unaffected by space flight (33). These results differed from previous results using spleen cells from rats flown on previous Cosmos flights (29). This may indicate a differential effect of space flight on cytokine production by lymphocytes located in different areas of the body. It is the first evidence for "compartmentilization" of the effects of space flight on immune responses.

All of the above studies have indicated that both short-term and long-term space flight can alter selectively cytokine production and action in test animals and humans. The full breadth of cytokine responses affected by space flight as well as the exact mechanism(s), duration, adaptation, reversibility, and significance to resistance to infection remain to be established.

POSSIBLE INTERACTIONS OF CYTOKINES IN THE REGULATION OF OTHER BIOLOGICAL SYSTEMS IN SPACE FLIGHT

Previously, the effects of other systems on the immune mechanism have been discussed. However, it is also possible that space flight-induced alterations of the immune response could alter these other biological functions as well.

An example of that is the interleukin-1/osteoclast activating factor response. This cytokine(s) has direct effects on the activation of osteoclasts that could destroy bone. Changes induced in the normal patterns of production of this cytokine could contribute to detrimental effects on skeletal function and structure seen after space flight.

Another example is the production of 1,25-dihydroxyvitamin-D_3 by macrophages. If

this is altered by space flight, then again the skeletal system could be affected drastically. Certainly, if the cytokine network fails during space flight and the host becomes susceptible to opportunistic infection, this puts the function of the entire host at risk.

It must be remembered that the immune system does not operate in isolation in space flight. All body functions work in an integrated fashion, and a change in one could affect drastically the entire body and the success of a space mission.

FUTURE DEVELOPMENTS WITH CYTOKINES IN SPACE

Much remains to be done before our understanding of the significance of space flight-induced changes in the cytokine network is complete. First and foremost, our understanding of the normal functioning and interaction of the cytokine network on the ground must be expanded. We are in our infancy of understanding the complex cytokine network. Many additional studies, therefore, need to be carried out on short and long-term space flights to fully understand the effects of flight on new developments in our understanding of the cytokine network.

An important step will be taken when we have a permanent laboratory in space where cytokine analyses can be performed. Presently, one drawback of working with the cytokine network in space is that we cannot carry out experiments directly in space, as fresh cells are required for cytokine production and action studies. Therefore, we cannot separate factors such as stress, acceleration, deceleration, and radiation from the effects of microgravity on the cytokine network. An argument can be made that during space flight, the host is exposed to all of these factors. Therefore we should study space flight as a single entity. This is true, but without a laboratory in space we will never be able to separate the different factors of space flight and learn the mechanisms of the effects of space flight on the cytokine network. Understanding those mechanisms will not only allow us to understand the breadth, duration,

reversibility (perhaps through application of exogenous cytokines), adaptation to, and effects on resistance to infection of the effects of space flight on cytokines, but may also yield new ways to explore the fundamental nature of the cytokine network itself. The future looks exciting and promising for the exploration of the cytokine network during space flight.

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FIGURE LEGENDS

Figure 1. Potential sites of interaction between space flight and the recognition phase of cytokine production/action.

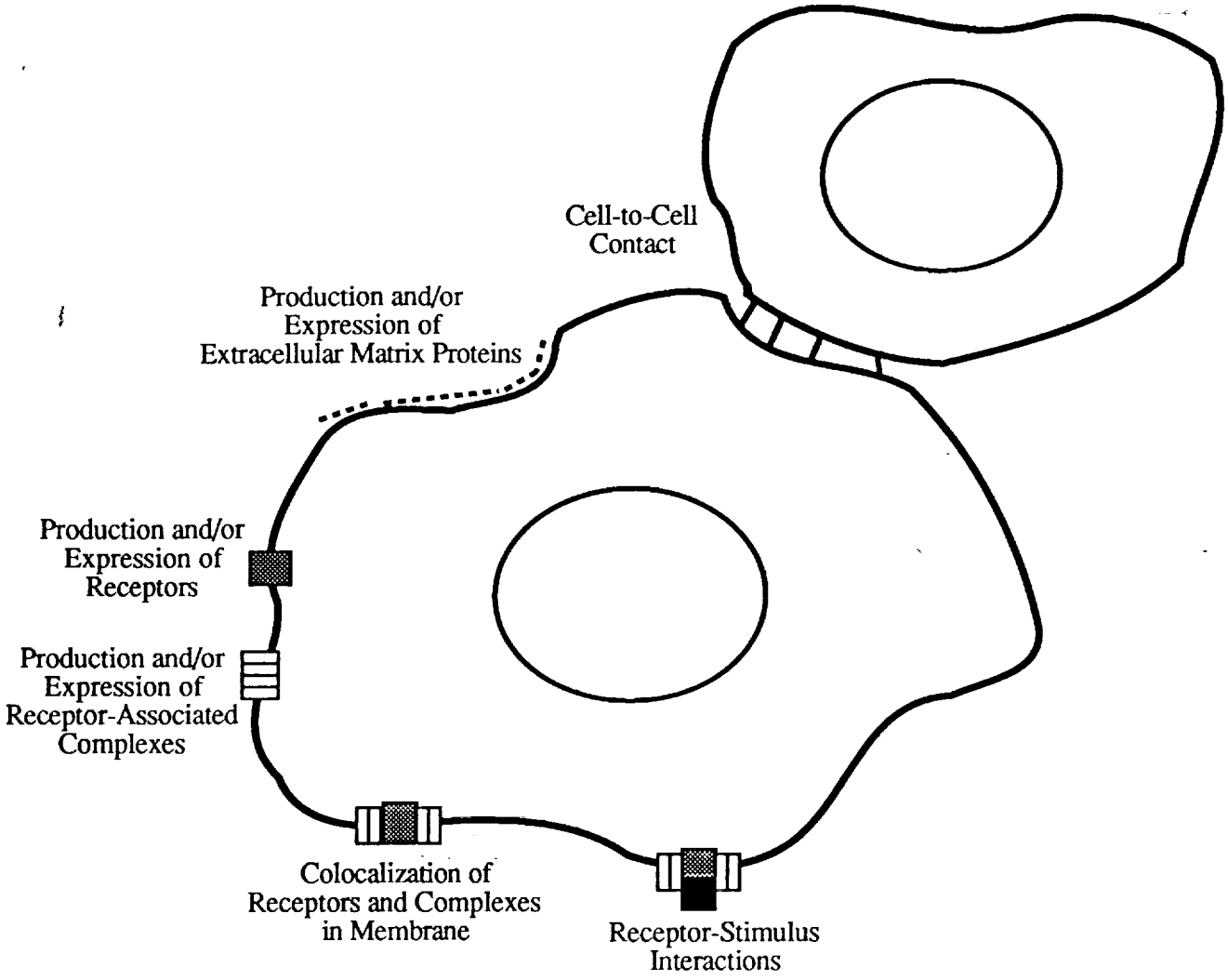
Figure 2. Potential sites of interaction between space flight and the effector phase of cytokine production/action.

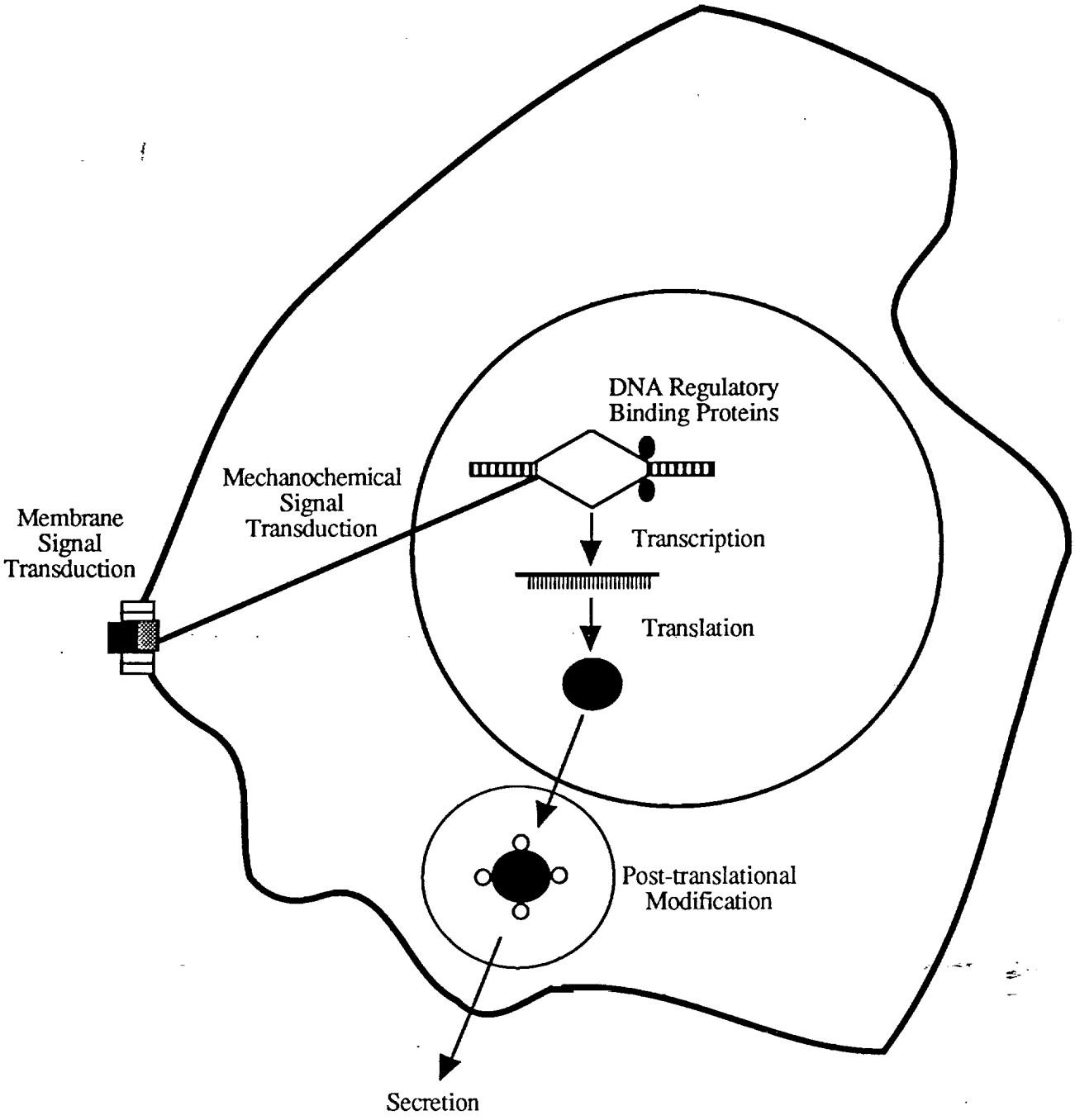
TABLE 1 - Ground-Based Models Affecting Cytokine Production/Action

Model	Cytokine Affected	Reference No.
Cell Culture - Poly-HEMA	Interferon-γ	12
Cave Isolation of Humans	Interferon-γ	13
Antiorthostatic Suspension of Rats	Interferon-α/β	18-22
	Interferon-γ	
	Interleukin-1	
	Interleukin-2	
	GM-Colony Stimulating Factor	

TABLE 2 - Space Flight Studies Affecting Cytokine Production/Action

Flight Condition	Cytokine Affected	Reference No.
Short-Term Flight of Leukocyte Cultures	Interferon-α/β	23-24
Leukocyte-Monocyte Cell Line - Short-Term Flight	Interleukin-1	25
	Interleukin-2	
Macrophage and Lymphocyte Cultures - Short-Term Flight	Interleukin-1	26,27
	Interleukin-2	
	Tumor Necrosis Factor-α	
	Interferon-γ	
Short-Term Flight of Humans	Interferon-α/β	23,24,28
Short-Term Flight of Rats	Interferon-α/β	22,29,
	Interferon-γ	31,32,33
	Interleukin-2	
	Osteoclast Activating Factor	
	M-Colony Stimulating Factor	
	GM-Colony Stimulating Factor	
Long-Term Flight of Humans	Interleukin-1β	29,30
	Interleukin-1	
	Interferon-α	
	Interferon-γ	


Cell-to-Cell
Contact
Production and/or
Expression of
Extracellular Matrix Proteins
Production and/or
Expression of
Receptors
Production and/or
Expression of
Receptor-Associated
Complexes
Colocalization of
Receptors and Complexes
in Membrane
Receptor-Stimulus
Interactions


DNA Regulatory
Binding Proteins
Mechanochemical
Signal
Transduction
Membrane
Signal
Transduction
Transcription
Translation
Post-translational
Modification
Secretion