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Editorial

Editorial: **Space flight modifies T cell activation—role of microgravity**

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Exposure of organisms or cells to spaceflight conditions has resulted in perturbations of immune responses [1–3]. Changes in the immune responses of humans, animals, and cell cultures have been observed [1–3]. Although cell-mediated immunity has been shown to be primarily affected, alterations in humoral immune responses after spaceflight have also been observed [1–3]. Both the natural and adaptive immune systems were affected [1–3]. Altered functions include cytokine production, leukocyte blastogenesis, NK cell and macrophage activity and production, antibody production, and enzyme functions in pathways important for immune functions [1–3]. The latest studies have indicated changes in in neutrophil, monocyte, and lymphocyte populations (cell population numbers and function), hypoplasia of lymphoid organs, altered expression of antibody variable heavy chain genes, and others in response to spaceflight conditions [4].

Although effects of the spaceflight environment on the immune system have been established for some time, two important questions have remained unanswered over time. The first is: what is the functional significance of the immune system changes that occur after spaceflight? The second is: what in the spaceflight environment is causing the changes observed in the immune system?

The functional significance of changes in immune responses remains to be answered. The immune response changes that occur during spaceflight could result in altered resistance to infection or cancer or to altered hypersensitivity reactions, yielding severe clinical manifestations that could endanger the host [1–3]. For the most part, this has not occurred in the spaceflights carried out to date [1–3]. As very long-term flights into space, including missions to Mars, are contemplated, however, this situation may change. Alterations in immune function, coupled with changes in growth patterns and expression of virulence factors induced by spaceflight [5], could result in increased risk of infection or cancer, as the duration of spaceflight increases, and return to earth for treatment becomes impossible. This should be the subject of future studies, and these studies will be required prior to the commencement of deep space exploration.

The study by Chang et al. [6] addresses the second question. The cause(s) of the alterations in immune function induced by exposure to spaceflight conditions have not been established. There are multiple variables encountered in the exposure of humans, animals, and cells to spaceflight conditions [1–4]. These include: microgravity (very reduced gravity experienced in low earth orbit spaceflights), stress (including landing), vibration, alterations in pressure and ambient temperature, radiation, changes in sheer forces, as well as other variables that may not, as yet, have been identified [1–4] (**Fig. 1**). These variables could each individually affect immune system function. Additionally, the variables could be interactive in the spaceflight environment to affect immune function in a way that does not normally happen on earth [1–4]. Hence, the question of which variable or variables are responsible for spaceflight-induced alterations in immune function had to be answered.

In the current study, human T cells were stimulated with Con A and anti-CD28 on board of the ISS to induce immune responses [6]. Microarray expression analysis after 1.5 h of activation demonstrated that the T cells activated in microgravity (during flight on the ISS) had distinct patterns of global gene expression that differed from those activated in a 1-g centrifuge during spaceflight (controlfor normal gravity that can be run during spaceflight) [6]. Forty-seven genes were identified that were significantly differentially down-regulated in T cells exposed to microgravity compared with T cells exposed to microgravity in a 1-g centrifuge [5]. Activation of Rel/NF-κB, CREB, and serum response factor gene targets, genes important in immune cell function pathways, was down-regulated [6]. These data suggest that the TNF pathway is a major early downstream effector pathway inhibited in microgravity, and this could lead to ineffective, proinflammatory host defenses against infections during long-term spaceflight.

These results may suggest that there could be a direct effect of microgravity on the expression of genes controlling immune cell function [1–4, 6]. Although several experiments have been carried out in the past to attempt to show a direct

Abbreviations: ISS=International Space Station

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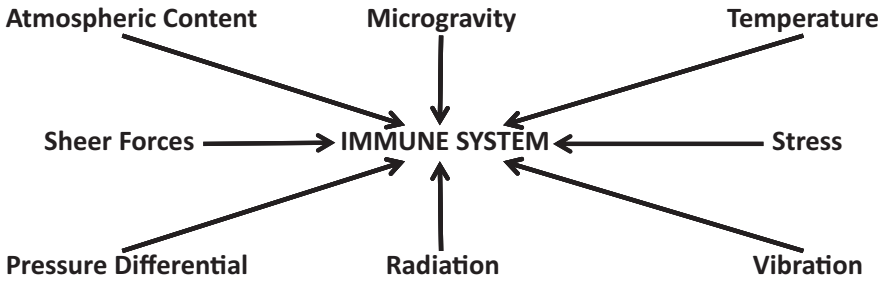

Figure 1. Multiple factors that occur during spaceflight that could influence the function of the immune system.

effect of microgravity on the immune system [7], these are the first convincing data using isolated cells and a positive 1-g control that were flown in space [1–4, 6]. The availability of new equipment has minimized interfering factors that compromised previous experiments [7]. It must be noted, however, that there still could be indirect effects of microgravity on immune response genes, as the centrifuge in space is not a perfect control.

Several questions remain to be answered. First, the current study has only shown effects on the transcription of genes that are important for immune cell function [5]. Future studies must confirm that there is a direct effect of microgravity on translation and therefore, on actual immune cell function. Second, it is unlikely that microgravity is the only variable that occurs during spaceflight that affects immune responses [1–4]. Other factors, such as radiation exposure and the stress response, are likely also to affect immune function [1–3]. The delineation of the role that the other variables that occur during spaceflight play in altering immune responses, as well as the possible interactions among the other variables and microgravity, remain to be established. Additionally, the transition of what happens to cells in culture to the whole organisms remains to be made [8]. Cells in culture may be more sensitive to microgravity effects than cells in situ in organs. Finally, as stated above, the clinical biomedical significance of any changes in the immune response induced by microgravity remains to be established.

In any case, the current study is a major step forward to indicate that microgravity exposure can directly affect immune responses [6]. The ramifications of these results for future safe exploration of space remain to be established.

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KEY WORDS:
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Editorial: **JQ1: giving HIV-1 expression a boost by blocking bromodomains?**

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In infected individuals, HIV-1 causes a decline in $CD4^+$ T cells, which is the etiological cause of the characteristic immunodeficiency seen in patients with AIDS. Although most infected $CD4^+$ T cells appear to die quickly ($t_{1/2}$=1 day), a small but significant number of infected cells are not killed. Instead, they revert to a quiescent state, turn off HIV-1 gene expression, and persist as long-lived memory T cells carrying latent HIV-1 genomes. The frequency of resting $CD4^+$ T cells that carry HIV-1 genomes is ~100 in 10^6. These cells comprise a library, called the latent reservoir, of previously circulating HIV-1 variants [1–3]. As a result of the extreme stability of this reservoir, individuals infected with HIV-1 must undergo lifelong treatment with HAART [4].

Adherence to drug regimens is critical for controlling viremia, and treatment interruptions can lead to rapid viral rebound, even though only 1% of the viral library consists of a replication-competent virus. Compared with earlier treatment regimens, the currently available antiretroviral drugs cause far fewer side effects and allow easier dosing in the form of combination pills. As a result, infected individuals can expect a higher quality of life with a near-normal life expectancy if treatment is started early and adherence is good. Still, there are patients for whom adherence is difficult due to circumstance, cost, or accessibility. In addition, the potential adverse effects of life-long HAART are, as yet, unknown. There is great impetus in the field, consequently, to find a cure, and much research is dedicated to eradication of the latent reservoir—the single greatest barrier to curing HIV-1 infection.

Unfortunately, latently infected cells are effectively indistinguishable from uninfected cells. Therefore, to purge the latent reservoir, all $CD4^+$ T cells that harbor replication-competent proviruses would have to be activated and then killed, either by viral cytopathic effects or immune effector mechanisms. Recent findings have shown that cytolytic T lymphocyte responses can be boosted with HIV-1-specific antigens to more efficiently kill infected cells, in which latency has been reversed [5]. Thus, the arguably larger challenge lies in activating only those cells containing a provirus, as global activation of $CD4^+$ T cells could trigger a fatal systemic immune response. There were early attempts to purge the latent reservoir and improve clinical outcomes by adding intermittent IL-2 to the treatment regimen of patients on HAART [6]; however, a rapid rebound in viremia occurred after treatment was interrupted [7].

In the years following, a connection emerged, linking the transcriptional activation of HIV-1 and the chromatin state of the LTR, which serves as the HIV-1 promoter [8, 9]. Notably, studies of the acetylation of HIV-1 Tat and of histones positioned at the LTR led to the discovery that HDAC inhibitors can strongly affect viral gene expression. Several drugs that target acetylation have been shown to reactivate latent HIV-1. Among these are trichostatin A, sodium valproate, and SAHA (Vorinostat), which are all HDAC inhibitors. Sodium valproate and SAHA have been tested in preliminary clinical trials (for review, see ref. [10]).

In this issue of the *Journal of Leukocyte Biology*, Banerjee et al. [11] examine the effect of a relatively new drug, JQ1, on reactivation of latent HIV-1 in cell culture systems. In contrast to other commonly used latency-reversing agents, JQ1 is not an HDAC inhibitor. This drug was developed originally as an antiproliferative and functions by binding to bromodomains [12], which are found in many well-studied acetyltransferases, such as p300 and p300/CREB-binding protein-associated factor, and interact specifically with acetylated lysines. JQ1 was discovered in a high-throughput screen of synthesized compounds that were predicted to fit into a central hydrophobic cavity shared by many bromodomains. To test the inhibitory effect of JQ1, a particular bromodomain-containing protein, Brd4, was used as a result of its possible role in NUT midline carcinoma and because it exhibited the highest binding specificity for JQ1 in its bromodomain protein family. Brd4 can decipher the histone code through interaction with acetylated histone tails [13]. As such, in the original study that characterized JQ1, addition of the compound to Brd4-dependent cell lines resulted in antiproliferative effects by displacing a Brd4 fusion oncoprotein from chromatin.

In concert with its interaction with histones, Brd4 also acts as a bridge between the mediator complex and P-TEFb, thereby recruiting P-TEFb to generic cellular promoters (**Fig. 1**) [14, 15]. P-TEFb is a heterodimer composed

Abbreviations: BD I/II=bromodomain I/II, CycT1=cyclin T1, HAART=highly active antiretroviral therapy, HDAC=histone deacetylase, P-TEFb=positive transcription elongation factor, Pol II=RNA polymerase II, SAHA=suberoylanilide hydroxamic acid, snRNP=small nuclear RNP

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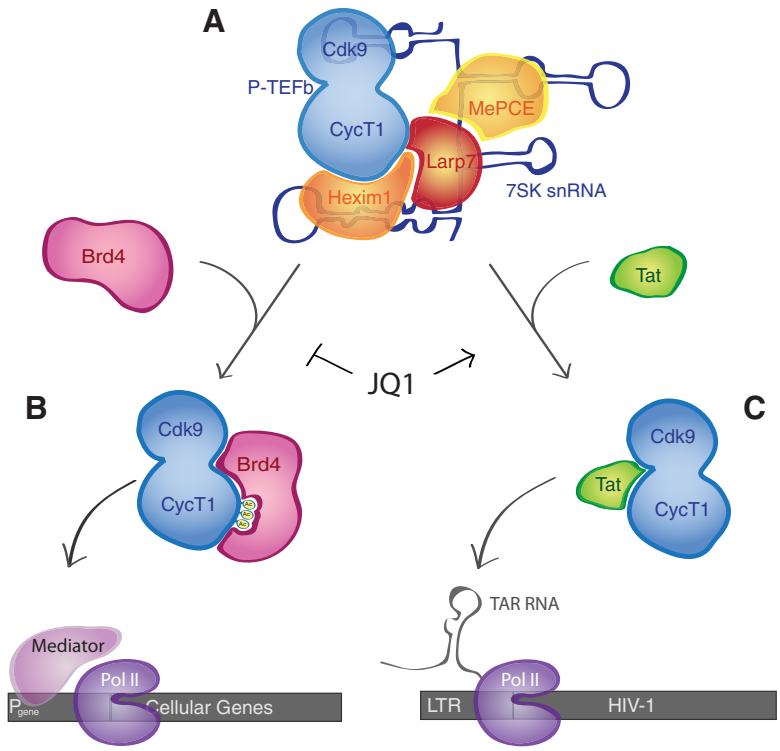

Figure 1. A parsimonious model of how JQ1 may reactivate HIV-1 expression. Half of cellular P-TEFb is in an inactive state as a complex with 7SK snRNA, Hexim1, La-related protein 7 (Larp7), and the methylphosphate capping enzyme (MePCE; A). The CycT1 subunit is acetylated by p300 (not shown), which permits Brd4 to bind P-TEFb and recruit it to general cellular promoters through its interaction with Mediator and chromatin (B). In the presence of JQ1, the interaction between Brd4 and P-TEFb is inhibited and, instead, Tat can bind P-TEFb and recruit it to the HIV-1 LTR through its interaction with the *trans*-activating region (TAR) RNA element (C).

of CycT1 and the kinase Cdk9. Generally, it exists in two major cellular pools: about half is in an inactive form (CycT1-Cdk9-7SK snRNP) sequestered from paused transcription initiation complexes, while the other half is in an active form (CycT1-Cdk9-Brd4) employed in activating target genes [14, 15]. The pivotal role of P-TEFb is to promote cellular transcription by phosphorylating the C-terminal domain of Pol II and converting Pol II into an elongating and, thus, processive enzyme. Accordingly, the interaction between Brd4 and P-TEFb is critical, as it is required for full transcriptional activation [16]. Brd4 binds the CycT1 subunit of P-TEFb through two domains. One interaction is via the Brd4 BD II, which binds triacetylated CycT1; the other interaction is through the P-TEFb-interacting domain, which is required for releasing P-TEFb from its repressive association with the 7SK snRNP [16].

The interaction between Brd4 and P-TEFb is of particular relevance to HIV-1, as P-TEFb is recruited to the viral LTR by the transactivator protein Tat. This recruitment is required for robust transcriptional activation of HIV-1; hence, Tat competes with Brd4 for binding to P-TEFb [14]. Consistent with competition between Tat and Brd4, Banerjee and colleagues [11] found that addition of JQ1 to several different HIV-1 reporter T cell lines resulted in an ~40-fold induction of viral transcription compared with mock treatment. To assess the specificity of JQ1 for targeting only transcriptional activation versus general T cell activation, the authors performed a microarray analysis. They compared changes in gene expression induced by JQ1 or αCD3/αCD28. Propitiously, the data indicated that JQ1 upregulated chromatin organization genes but down-regulated lymphocyte activation genes. This is desirable for a treatment where general T cell activation should be avoided.

This study of JQ1 raises many interesting questions. One could imagine that JQ1, a molecule that abrogates the normal cellular function of Brd4, may increase the pool of P-TEFb that is free for Tat engagement and consequently promote reactivation of HIV-1 expression (Fig. 1). It would be interesting to see how JQ1 affects the distribution of P-TEFb between its inactive and active forms. Also unknown is the effect of JQ1 on the binding between Brd4 and P-TEFb. Brd4 binds P-TEFb through BD II, but JQ1 has a higher affinity for BD I (K_d ~50 nM for BD I; K_d ~90 nM for BD II). In the experiments of Banerjee and colleagues [11], experimental concentrations were 500 nM; assuming the cellular drug concentrations were in the same range, it is possible that both BD I and II were occupied by JQ1. Do both bromodomains need to be bound by drug to see the effect? Another interesting aspect of this drug is the prospect of its synergy with HDAC inhibitors; if JQ1 does act by increasing the cellular pool of free P-TEFb, then the concomitant use of HDAC inhibitors, which promote an open chromatin environment at the HIV-1 LTR, could lead to stronger reactivation than either drug treatment alone. Precisely how JQ1 increases HIV-1 expression in this experimental system is still unclear, as is whether Tat and Brd4 are even involved in this effect. Nevertheless, the current study of JQ1 may very well open up new approaches for reactivating latent HIV-1 and ultimately curing the infection.

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KEY WORDS:
latency · P-TEFb · Brd4 · HDAC inhibitor

Editorial: **Yin-Yang of EP receptor expression**

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PGE_2 is a potent lipid mediator that is produced and released by multiple cell types in response to inflammatory stimuli. Production of PGE_2 from arachidonic acid involves the sequential synthesis of PGG_2/PGH_2 by the two COX enzymes (COX-1 and COX-2), followed by the synthesis of PGE_2 by mPGES-1 and mPGES-2 and cytosolic PGE_2 synthase. Of these, it is mPGES-1 that is most associated with inflammation. PGE_2 displays pro- and anti-inflammatory functions reflecting its ability to interact with four distinct GPCRs (EP1–4), each having various activating or inhibitory functions.

The incidence of asthma and allergic diseases, including allergic rhinitis, acute urticaria/angioedema, and atopic dermatitis, has been increasing over the past 25–50 years. Whereas differences exist among these diseases, one common feature is that all involve, at least in part, the action of mast cells (and basophils) by antigen-bound IgE signaling through the FcεRI receptor. Mast cells are considered the central player in these IgE-mediated reactions, and engagement of IgERs results in the release of various granules, cytokine, and lipid products (**Fig. 1**). Several studies have demonstrated a direct role for PGE_2 acting through the EP2 receptor in preventing mast cell activation [1, 2], whereas other studies have indicated that signaling through EP3 is considered proinflammatory [2, 3]. In this issue of the *Journal of Leukocyte Biology*, Serra-Pages and colleagues [4] demonstrate that the ratio of EP2 to EP3 receptors on the surface of a mast cell influences the activation potential of these cells when FcεRI is stimulated in a PGE_2-containing milieu. In examination of various mast cell lines, the authors found that those with high levels of EP2 could suppress FcεRI activation in the presence of PGE_2, but when EP3 levels were high, FcεRI activation of mast cells was enhanced. EP2 engagement was associated with an increase in cAMP and inhibition of calcium flux. The importance of the EP2/EP3 ratio is amplified by the demonstration that EP3 is a higher affinity receptor for PGE_2 binding than is EP2. These results suggest a generalized model in which the ratio of EP2 to EP3 determines the activation potential of a mast cell when activated through FcεRI (Fig. 1).

Abbreviations: AERD=aspirin-exacerbated respiratory disease, CysLT=cysteinyl leukotriene, mPGES-1/2=microsomal PGE2 synthases 1/2, NP=nasal polyp

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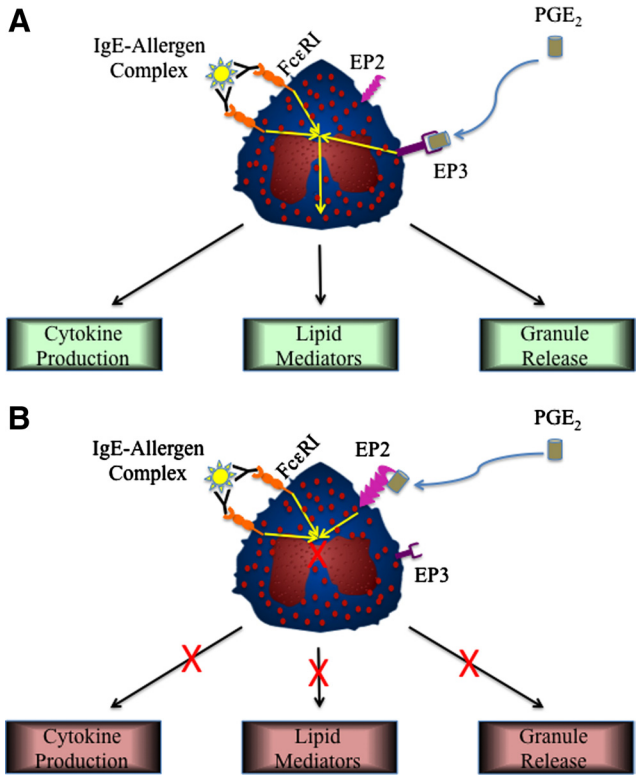

Figure 1. FcεRI activation of mast cells and modulation by PGE_2. (A) On mast cells with high-affinity EP3 expression (low EP2/EP3 ratio), PGE_2 enhances activation of mast cells following cross-linking of FcεRI by allergen-bound IgE, leading to release of cytokines, lipid mediators, and granule products. (B) On mast cells with high EP2 expression (high EP2/EP3 ratio), PGE_2 suppresses activation of mast cells following cross-linking of FcεRI by allergen-bound IgE, blocking release of cytokines, lipid mediators, and granule products.

Of major interest are the implications of alterations in EP receptor expression to AERD, which is a syndrome characterized by asthma, hyperplastic eosinophilic sinusitis with NP formation, the usual absence of atopy, and intolerance to aspirin and other NSAIDs [5]. A central feature of AERD is its association with profound overproduction and overresponsiveness to CysLTs and a decrease in PGE_2 production and responsiveness. It is the up-regulation of CysLT synthesis pathways that underlies the observed life-threatening surge in CysLT secretion and subsequent anaphylactic reaction following ingestion of aspirin or other NSAIDs in this disease [6]. The key is that these reactions are not mediated through FcεRI but do involve activation of mast cells and eosinophils. PGE_2, acting through EP2 receptors, is able to block this eosinophil and mast cell degranulation from non-AERD subjects. Critical to the pathogenesis of AERD is the observation that patients with AERD constitutively display low levels of PGE_2 [7]. COX-2 mRNA and protein expression are also diminished in NPs of subjects with AERD [7, 8]. With this relative absence of COX-2, AERD subjects become dependent on COX-1 for the PGE_2 that is necessary to restrain mast cell and eosinophil activation. The reduced capacity to synthesize PGE_2 contributes to the severity of inflammation observed in AERD and accentuates the sensitivity of these individuals to the inhibition of PGE_2 synthesis associated with COX-1 inhibition by aspirin and other NSAIDs. This sensitivity is amplified further by the reduced expression of the anti-inflammatory EP2 receptors also observed in this condition [9]. Inhalation of PGE_2 into the airways protects against these non-IgE-mediated reactions, presumably by targeting EP2 receptors on airway smooth muscle [10]. The low EP2/EP3 ratio on mast cells likely contributes to this disease, as any PGE_2 that is available would signal through the EP3 receptor and activate these cells, contributing to the proinflammatory cascade. Strategies specifically targeting the EP2 receptor may mitigate this response and slow or reverse disease progression.

Whereas the study by Serra-Pages et al. [4] is intriguing, many questions remain unresolved. Their study focused on EP receptor levels in mast cells and modulation of FcεRI-dependent activation; however, EP receptors are expressed on numerous cells involved in the inflammatory response, and as discussed for AERD, not all influences are mediated through FcεRI. Studies are therefore needed to examine the EP2/EP3 ratio on other cells, including eosinophils, basophils, monocytes, and T cells to determine whether high EP2 receptor levels will suppress activation of these cell types, as was observed in mast cells. Is the EP2/EP3 ratio important in other diseases, and if so, is the ratio stable, or does it fluctuate as the disease progresses? Similarly, is a healthy individual's EP receptor ratio fixed? One must also consider the differences in receptor affinity. The EP3 receptor has higher affinity for PGE_2 than does the EP2 receptor. Simply changing the EP2 receptor level may not be enough to offset the higher affinity of the EP3 receptor. It is therefore likely that there is a critical point where the higher EP3 affinity can be overcome by increased numbers of EP2 receptors. This point has yet to be determined. It is also important to consider that in other diseases and on other cell types, the EP1 and EP4 receptors may also contribute to the observed response to PGE_2. Understanding these questions will aid in development of therapies targeting these pathways.

Tipping the EP receptor balance in many diseases, whether they are involved in influencing FcεRI signaling, offers an attractive strategy for therapeutic intervention. Agonists directed at the EP2 receptor could lead to suppression of proinflammatory products produced by mast cells but also numerous other cell types. In diseases where the EP2 receptor levels are low, this may not be effective. Instead, antagonists directed at

the higher-affinity activating EP3 receptor may prove more efficacious. These issues need to be sorted out but do suggest a promising area for controlling the inflammatory component associated with chronic inflammatory diseases.

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