Spaceflight and development of immune responses

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Spaceflight and development of immune responses. J. Appl. Physiol. 85(4): 1429–1433, 1998.—The NIH.R1 Space Shuttle experiment was designed to study the effects of spaceflight on rodent development. Pregnant rats were flown on the Space Shuttle for 11 days, and pregnant control rats were maintained in animal enclosure modules in a ground-based chamber under conditions approximating those in flight. Additional controls were in standard housing. The effects of the flight on immunological parameters of dams, fetuses, and pups were determined. Blastogenesis of spleen cells in response to mitogen was inhibited in flown dams but was not inhibited in cells from their pups. Interferon-γ production by spleen cells showed a trend toward inhibition in flown dams but not in their pups. The response of bone marrow cells to colony-stimulating factor showed a trend toward inhibition after spaceflight in dams, but the response of fetus and pup liver cells was not inhibited. Total serum IgG was not affected after spaceflight in dams, but the response of fetus and pup spleen cells showed a trend toward inhibition. The response of bone marrow cells to exogenous colony-stimulating factors (CSF) (25–27), and alterations in leukocyte subset distribution (2, 20, 25–28) were determined to begin the establishment of the effects of spaceflight on development of the immune system.

MATERIALS AND METHODS

Animals and Spaceflight Conditions

Pregnant, specific pathogen-free, female rats that had tested positive for sperm were shipped via air freight from Taconic Farms (Germantown, NY) to the Kennedy Space Center, FL, on day 2 of gestation. All animal care and use procedures were approved by the Institutional Animal Care and Use Committees of the National Aeronautics and Space Administration (NASA) Kennedy Space Center and the NASA Ames Research Center, and receipt of tissue was approved by the Institutional Animal Care and Use Committee of the Carolinas Medical Center according to guidelines of the National Institutes of Health and US Department of Agriculture. On day 7 of gestation, pregnancy was confirmed after isoflurane anesthesia (Abbott Laboratories, North Chicago, IL) by counting and recording the number of decidual swellings on each uterine horn (3, 13). On day 8 of gestation, 10 pregnant rats were placed into NASA animal enclosure modules (AEM; 5 rats per cage) and loaded on to the middeck of the Space Shuttle. The Space Shuttle was launched into orbit when the rats were in day 9 of gestation (3, 13). The Shuttle landed at Edwards Air Force Base in California after an 11-day flight with the rats in day 20 of gestation. About 3 h after landing, flight animals were recovered, anesthetized with isoflurane, and then subjected to unilateral hysterectomy to provide for study of fetal material. The dams were then allowed to recover and deliver vaginally. There were three groups of 10 animals each maintained at the Kennedy Space Center. The first group (AEM control) was a 24-h-delayed synchronous control group that received the same surgeries as the flight group and was housed (5 animals per cage) in AEMs and exposed, as closely as possible, to all flight conditions except microgravity (3, 13). Vivarium control group A was housed in standard rat caging without any surgery. The second vivarium control group (group B) received a unilateral hysterectomy on day 20 of gestation, and rats were housed in standard rat caging. There were no significant differences in body mass noted among vivarium control group B, flight, and synchronous control rat groups on the day the flight group was loaded on the Space Shuttle (3, 13).
The flight animals all delivered pups vaginally between 54 and 58 h after recovery from the Space Shuttle, whereas AEM control animals delivered pups between 48 and 75 h after simulated recovery. The mean numbers of pups born vaginally for each group were flight, 5.80 ± 2.04 (SD); AEM, 5.70 ± 1.16; vivarium control group A, 5.8 ± 1.88; and vivarium control group B, 10.9 ± 11.7 (this group had no unilateral hysterectomy). There were no significant differences in the number of pups among flight, AEM, and vivarium control groups. Mean weights of pups per group were flight, 5.66 ± 0.44 g; AEM, 5.83 ± 0.41 g; vivarium control group B, 6.92 ± 0.38 g; and vivarium control group A, 6.22 ± 1.7 g. Mean weights of pups from flight and AEM control groups were not statistically different; however, both flight and AEM groups had a statistically significant lower weight than did vivarium controls. Information regarding pup numbers and weight was obtained courtesy of Dr. Hubert Burden of East Carolina University.

Blood and Tissue Sampling

At ∼3 h after delivery of pups, dams were anesthetized with isoflurane, and blood was withdrawn from the abdominal aorta. Then spleens were removed and placed in RPMI-1640 medium with 10% fetal bovine serum (FBS), HEPES buffer, antibiotics, and sodium bicarbonate (GIBCO, Grand Island, NY). The epiphyses were cut from the humerus, which was then flushed with supplemented RPMI-1640 medium to obtain bone marrow. Immediately after birth and euthanasia by guillotine, blood, spleens, and livers were obtained from one to three fetuses and/or pups per dam. These tissues were placed in supplemented RPMI-1640 medium. All samples were maintained at 40°C and shipped via air express for 24 h later at the Carolinas Medical Center. Cells from each individual dam, pup, and fetus were placed through all experimental procedures individually.

Determination of Immunological Parameters

Due to the limitations of spaceflight experimentation, including limited numbers of animals, limited sample sizes and volumes, and the need to design experiments to allow for maximum utilization of shared animals, resources were not available to allow for titrations to determine the number of cells used for assays and concentration of mitogens and cytokines. Therefore, the numbers of cells used for assays and the doses of mitogen and cytokines used were based on results of previous studies (16, 19, 26, 27) and preliminary experiments indicating that use of those doses would be successful.

Leukocyte blastogenesis. Splenocytes from all animals were treated as follows to carry out the blastogenesis assay (19).

The cells were washed once in supplemented RPMI-1640 medium, counted, and resuspended in supplemented RPMI-1640 medium at a concentration of 2 × 10^6 cells/ml. Cells were distributed in 96 flat-bottom-well plates (Falcon; Becton-Dickinson, Lincoln Park, NJ) at 2 × 10^5 cells/well and cultivated in the presence of supplemented RPMI-1640 medium with or without concanavalin-A (Con-A; 5 µg/ml; Sigma, St. Louis, MO). After 30 h of incubation (37°C, 5% CO2), 1 µCi of tritiated thymidine (Amersham, Arlington Heights, IL) was added to each well. After an additional 18-h incubation, cells were harvested onto glass filter paper by using a semiautomated cell harvester (Cambridge Technology, Watertown, MA). Uptake of thymidine was measured by using a liquid scintillation counter (Packard, Meridian, CA).

IFN-γ assay. Levels of IFN-γ were determined in supernatant fluids of spleen cells placed in 96-well culture dishes (3 × 10^6 cells/ml) and challenged with 5 µg/ml Con-A (8, 16). The mitogens were purchased from Sigma Chemical. Cultures were incubated for 48 h at 37°C in 5% CO2. After harvest of the supernatant fluids, IFN-γ levels were determined by using rat IFN-γ ELISA kits (Biosource International, Cuperino, CA) following the manufacturer's recommendations. The plates were read at 450 nm by using an ELISA plate reader (Dynatech, Chantilly, VA). IFN-γ levels (expressed in pg/ml) were derived by comparison with standard curves determined along with the samples.

CSF assay. For dams, 1 × 10^6 bone marrow cells/ml from the humerus were resuspended in a 2% methylcellulose solution prepared in supplemented McCoy's 5-A medium (GIBCO) containing 30% FBS (25, 26). For fetuses and pups, 1 × 10^5 individual liver cells were placed in the medium. Medium for experimental group cultures contained a concentration of 40 ng/ml recombinant murine granulocyte macrophage-CSF (GM-CSF; Genzyme, Cambridge, MA), which cross-reacted with rat cells (26, 27). For each animal tested, five 35-mm tissue culture dishes, each containing 1 ml of the bone marrow cell suspension, were set up for control (−CSF) and for experimental (+CSF) groups. Dishes containing surviving cells were placed in a 37°C incubator with 5% CO2 (26, 27). After 7 days of incubation, we evaluated 10 microscope fields from each petri dish for the number of colonies formed; a colony represented aggregates of 50 or more cells (25, 26).

Determination of IgG titers. We standardized an ELISA procedure to determine concentrations of IgG in the serum. To generate a standard curve, a stock preparation of purified goat anti-rat IgG (Sigma) was prepared at 5 µg/ml in carbonate-bicarbonate buffer and diluted 1:4 to a final concentration of 5 µg/ml. The wells of Immunon-1 ELISA plates were coated with 50 µl of each concentration of IgG. The plates were incubated overnight under tissue culture conditions, then washed four times with PBS. To each well, 250 µl of ELISA block were added (PBS containing 0.1% Tween-80 (PBST) and 1.0% bovine serum albumin). After 90 min of incubation, the ELISA block was removed, followed by four washes with PBST. Serum from each dam and pup was thawed rapidly at 37°C and diluted 1:10, 1:100, and 1:1,000 in PBST. To each well, 250 µl of ELISA block were added (PBS containing 0.1% Tween-80 (PBST) and 1.0% bovine serum albumin). After 90 min of incubation, the ELISA block was removed, followed by four washes with PBST. Serum from each dam and pup was thawed rapidly at 37°C and diluted 1:10, 1:100, and 1:1,000 in PBST. To each well, we then added 50 µl of rabbit anti-rat IgG peroxidase conjugate diluted in PBST. After 60 min of incubation, the wells were washed four times with PBS. To each well, we then added 50 µl of o-phenylene-diamine-dihydrochloride and H2O2 substrate in citrate phosphate buffer. After 40 min of incubation, 50 µl of 2.5 N H2SO4 were added to each well. The plates were then read in a Dynatech MR5000 plate reader at a wavelength of 490 nm. From this, a standard curve of optical density 490 nm vs. IgG concentration was generated for each assay. For each sample, serum was added to four duplicate wells at each dilution (1:10, 1:100, and 1:1,000).

Statistical Analysis

For all experiments except the antibody titration, statistical analysis was performed by using ANOVA. For the antibody titration, statistical analysis was performed by using a nonparametric Mann-Whitney U-test. In all cases, alpha was set a priori at P < 0.05.

RESULTS

Con-A-Stimulated Splenocyte Blastogenesis of Splenocytes from Dams and Pups After Spaceflight

Con-A-induced blastogenesis was significantly inhibited when splenocytes from flight group dams were challenged after flight compared with blastogenesis of
that had been flown during group (Table 1). Blastogenesis of splenocytes from pups compared with spleen cells from the AEM group was not significantly altered compared with the AEM control; however, blastogenesis of splenocytes from vivarium group B was significantly increased compared with levels in controls from the AEM group. (Table 1).

Table 1. Concanavalin-A-stimulated blastogenesis of splenocytes from rat dams and pups after spaceflight

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<td>n</td>
<td>Group A</td>
<td>Group B</td>
<td>AEM</td>
<td>Flight</td>
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<tr>
<td>Dam</td>
<td>10</td>
<td>40,434±10,413</td>
<td>93,618±17,623†</td>
<td>72,589±18,309</td>
<td>16,750±3,825*</td>
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<tr>
<td>Pup</td>
<td>10</td>
<td>55,612±12,322†</td>
<td>28,302±11,482</td>
<td>24,567±6,917</td>
<td>34,452±8,951</td>
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Values are means ± SE in counts/min; n = no. of animals. Cells from 1 to 3 pups from each of the 10 dams were used in the experiment. Cell samples were not pooled but tested independently. AEM, animal enclosure module. *Statistically significant decrease compared with AEM group; †statistically significant increase compared with AEM group.

spleen from AEM control rats and spleen cells from rats from vivarium group B showed significantly increased blastogenesis compared with spleen cells from the AEM group (Table 1). Blastogenesis of splenocytes from pups that had been flown during days 9–20 of gestation was not significantly altered compared with the AEM control; however, blastogenesis of splenocytes from vivarium group A rats was significantly increased compared with that of controls (Table 1).

Con-A-Stimulated IFN-γ Production by Splenocytes of Dams and Pups After Spaceflight

Compared with blastogenesis of spleen from AEM control rats, when splenocytes from space flight groups dams were challenged after flight, Con-A-induced IFN-γ production showed a trend toward inhibition (Table 2). However, vivarium group A conditions produced a significant increase in IFN-γ production. IFN-γ production of splenocytes from pups that had been flown during days 9–20 of gestation was not significantly altered compared with levels in controls from the AEM groups, but vivarium group A conditions appeared to produce a significant increase in IFN-γ production (Table 2).

GM-CSF-Stimulated Colony Formation of Dam, Pup, and Fetus Cells After Spaceflight

In comparison with the AEM control group, GM-CSF-stimulated colony formation of bone marrow cells in dams showed a trend toward inhibition when cells obtained from the flight group after spaceflight were tested (Table 3). However, liver cell colony formation was not inhibited, by comparison of cells from the pup and fetus flight groups with AEM controls (Table 3).

Table 2. Concanavalin-A-stimulated IFN-γ production by splenocytes of rat dams and pups after spaceflight

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<td>Dam</td>
<td>10</td>
<td>4,667±1,438*</td>
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<tr>
<td>Pup</td>
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<td>6,519±2,254*</td>
<td>577±295</td>
<td>536±537</td>
<td>146±123</td>
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Values are means ± SE in pg/ml; n = no. of animals. IFN-γ interferon gamma. Cells from 1 to 3 pups from each of 10 dams were used in the experiment. Cell samples were not pooled but tested independently. *Statistically significant decrease compared with AEM group.

Serum IgG Titers of Dams and Pups After Spaceflight

Serum IgG titers were not altered in any flight group dams or pups compared with all control groups (Table 4).

DISCUSSION

In the present study, pregnant rats were flown on the Space Shuttle for 11 days just before the end of the gestation period for the rats. The pregnant rats were launched into space on day 9 of gestation and returned on day 20. Some of the offspring were removed by cesarean section just before birth and were evaluated as fetuses. Other offspring were allowed to be born at term and were evaluated as pups. All offspring of flown rats were apparently, overall, in healthy condition when born.

Operational requirements for all of the Space Shuttle flight experiments required the flight period to be between days 9 and 20 of gestation. The flight continued for the maximum time available for this particular Space Shuttle mission (11 days); the landing occurred close to the end of the gestation period, with enough margin to ensure pregnant rats would not deliver in flight. Additionally, the second half of the gestation period is the time when major development and maturation of the immune parameters that we observed occur, and maturation of the immune response could have been very much affected during the time interval we observed (22–24).

The immunological parameters tested for the parent dams flown in space were similar to those reported previously for rats flown in space (2, 4, 7–12, 14–16, 19–21, 25–32). These immune parameters, leukocyte blastogenesis, IFN-γ production, and the response of bone marrow cells to GM-CSF have all been shown to occur by 10.220.33.3 on July 12, 2017 http://jap.physiology.org/ Downloaded from

Table 3. GM-CSF-stimulated colony formation of rat dam, pup, and fetus cells after spaceflight

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<tr>
<td>Dam</td>
<td>10</td>
<td>32±8</td>
<td>41±6</td>
<td>37±10</td>
<td>21±6</td>
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<tr>
<td>Pup</td>
<td>10</td>
<td>19±2</td>
<td>17±3</td>
<td>12±3</td>
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<tr>
<td>Fetus</td>
<td>10</td>
<td>ND</td>
<td>16±2</td>
<td>15±1</td>
<td>13±2</td>
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Values are means ± SE in no. of colonies per plate; n = no. of animals. GM-CSF, granulocyte macrophage colony-stimulating factor. Cells from 1 to 3 pups and fetuses from each of the 10 dams were used in the experiments. Cell samples were not pooled but tested independently. ND, not done.
be altered during and after short-term spaceflight (8–11, 19, 20, 25–27, 30, 32). Total antibody levels have been shown not to be altered after short-term spaceflight and were chosen as a negative control (33). Generally, compared with AEM controls, which most closely imitate spaceflight conditions, leukocyte blastogenesis (10, 11, 19, 20, 30, 32), IFN-γ production (8), and the response to GM-CSF (9, 25–27) were all inhibited in the dams after spaceflight. Not all differences were statistically significant; this may be accounted for by the relatively small sample size. Leukocyte blastogenesis and IFN-γ production were shown to be enhanced compared with AEM controls under some vivarium conditions. This could have been due to the wide variability seen in the vivarium assays as well as the relatively small number of animals in each group. In any case, these increases could not have contributed to the inhibition seen after spaceflight. Serum Ig levels have been reported to be unaffected after short-term spaceflight in the Space Shuttle (33). Stress has been postulated to play a role in the effects of spaceflight on immune responses (28, 32), and stress has been shown to depress immune responses (1, 5, 6, 18). There is widespread evidence that exposure of animals to a wide variety of stressors (including mild electric shock or metabolic and psychological stressors) can alter a wide variety of cell-mediated immunological parameters, including cytokine production and leukocyte blastogenesis (1, 5, 6, 18). It is possible that a stress component occurred during spaceflight that could have contributed to the depression of IFN-γ production observed in the dams flown in space.

Additionally, the surgery and anesthesia carried out on the dams could also have contributed to the alterations in immune responses seen in both the dams and the offspring after spaceflight. However, the fact that patterns of immunological changes similar to those observed in the dams have been observed earlier, when similar anesthesia was not used and no survival surgery was performed, suggests that surgery and anesthesia had minimal effects on the immune responses of the dams (8–11, 19, 20, 25–27, 30, 32). The influence of surgery on lymphocytes and immunoresponse could explain differences in immunological parameters between pups in different vivarium groups, but, again, because there was no difference in immunological parameters observed between flight and AEM control groups, it is unlikely that surgery and anesthesia influenced the lack of spaceflight effects that were observed in immune parameters of pups and fetuses.

Table 4. Serum IgG titers of rat dams and pups after spaceflight

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<td>n</td>
<td>Group A</td>
<td>Group B</td>
<td>AEM</td>
<td>Flight</td>
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<tr>
<td>Dam</td>
<td>10</td>
<td>642 ± 33</td>
<td>677 ± 51</td>
<td>628 ± 53</td>
<td>596 ± 64</td>
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<tr>
<td>Pup</td>
<td>10</td>
<td>616 ± 37</td>
<td>635 ± 47</td>
<td>662 ± 49</td>
<td>588 ± 71</td>
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Values are means ± SE in mg/dl. Blood from 1 to 3 pups and fetuses from each of the 10 dams was used in the experiment. Blood samples were not pooled but tested independently.

None of the pups or fetuses (analyzed where possible) showed an alteration in any of the observed immunological parameters after spaceflight. Leukocyte blastogenesis, IFN-γ production, the response to CSF-GM, and serum IgG levels of offspring exposed to spaceflight in utero were not altered. Again, leukocyte blastogenesis and IFN-γ production were found to be enhanced in some vivarium conditions when compared with AEM controls.

However, the present results do not indicate that spaceflight has little or no effects on the development of immunological parameters in rats beyond the conditions of the present experiment. It must be noted that spaceflight for the pregnant dams commenced at 9 days postimpregnation, long after the initiation of crucial events in pregnancy and implantation (2, 10). It may be that commencement of spaceflight before conception or earlier in gestation would have had very different effects on the development of immunological parameters. Additionally, longer-term spaceflights could have effects not observed during the present study. Additional experimentation will, therefore, be required before a complete answer can be obtained concerning the effects of spaceflight on the development of the immune system.

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