Cantaurus, Vol. 2, 48-51, May 1994

Psychosocial Environment Change and its Effect on the Immune Response of Mice

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Abstract

Numerous studies done on the effects of differential housing on the immune responses of rodents have produced conflicting results. This study was done in an attempt to clarify some of the differences in these results. In some studies the group-housed animals have higher immune responses while in other studies isolation-housed animals have the better immune responses. Mice were differentially housed for 6 weeks. Twenty mice were housed individually and twenty were housed in groups of ten. Blood was taken each week and total white blood cell (WBC) counts were done on each sample with a hemocytometer to monitor their immune systems. Four days before blood was taken in the seventh week, all mice were injected with chicken ovalbumin, acting as an antigen. At this time ten of the group housed mice (G) were put into individual housing and ten of the individually housed mice (I) were put into group housing. Once again blood was drawn to observe if immune response was affected by the change in housing conditions. The only significant difference showed that all mice that ended up in group housing when blood was taken in the seventh week had stronger immune responses with regards to total WBC count ($p=0.013$). The counts were 14,908 for I to G, 12,992 for G to G, 10,764 for I to I, and 12,389 for G to I. The hypothesis was that the change in housing conditions would influence an immune response more than the housing conditions of isolation or grouping. The evidence, however indicates that group-housing is more conducive to stimulation of the immune system.

Introduction

It is recognized as central dogma in the field of stress biology that isolation housing produces major behavioral and physiological changes, especially neuroendocrine changes in the rodent. Isolated mice have been observed to exhibit a more aggressive nature in studies of aggression and fighting. Also, isolated mice have stronger adrenocortical reactivity (Brain, 1975) and long-term isolated rats have heavier adrenals and thyroid and lighter spleen and thymus (Hatch et al, 1963). Because isolated housing causes more changes than the "normative" conditions of group housing, the term "isolation stress" (Hatch et al, 1963) has come into common use. This term is still used, but there are now questions as to whether or not isolated housing should be considered a stress.

Studies have been done comparing immune responses of differentially housed rodents using various immune parameters. In general, it has been shown that isolation-housed mice have a higher immune response compared to group-housed mice. Individually housed mice have been shown to have higher specific immune responses and higher macrophage activity than group housed mice (Hoffman-Goetz, et al, 1992). When infected with the protozoan *Plasmodium berghei*, group-housed mice showed less of a resistance to the malaria infection than did the individually housed mice (Stein et al, 1989). The same occurred with mice that were exposed to *Mycobacteruim tuberculosis*, only the mice went through a change in housing prior to exposure. Those mice that were previously in group housing then changed to individual housing had a higher resistance than the mice that were initially housed individually and then moved into group housing (Rabin, et al, 1989).

Hormonal assays have shown that isolated mice had greater amounts of plasma corticosterone following ACTH administration compared to group housed mice. In general, adrenocortical reactivity is higher in those mice in isolation housing (Brain, 1975). Glucocorticoids are part of this adrenocortical reactivity and a deficiency in them makes the patient more susceptible to stress. Grouped housed mice have also been found to have lower titers of circulating antibodies than isolated mice (Stein et al, 1976).

Although much of the evidence leans toward isolation housing as beneficial for the immune system, there are studies that oppose this generalization. In response to bovine serum albumin individually housed mice have lower precipitin titers than those housed in groups (Glenn and Becker, 1969). Tumor growth has been found to be reduced in isolated mice (Dechambre and Gosse, 1973), and yet another study found that in another strain, group housed mice developed tumors at a later age (11.9 months) than isolated mice (9.6 months) and only 80% of the group housed mice developed tumors versus 98% of the individually housed mice (Rabin et al, 1989).

The immune parameters as well as strains of rodents are many and there is varying evidence as to which is a stress on rodents: group housing or isolation housing? In some studies it was clear that the animals were given time to acclimate to their housing condition, yet not all papers indicated this allowance. Therefore, the proposal in this research

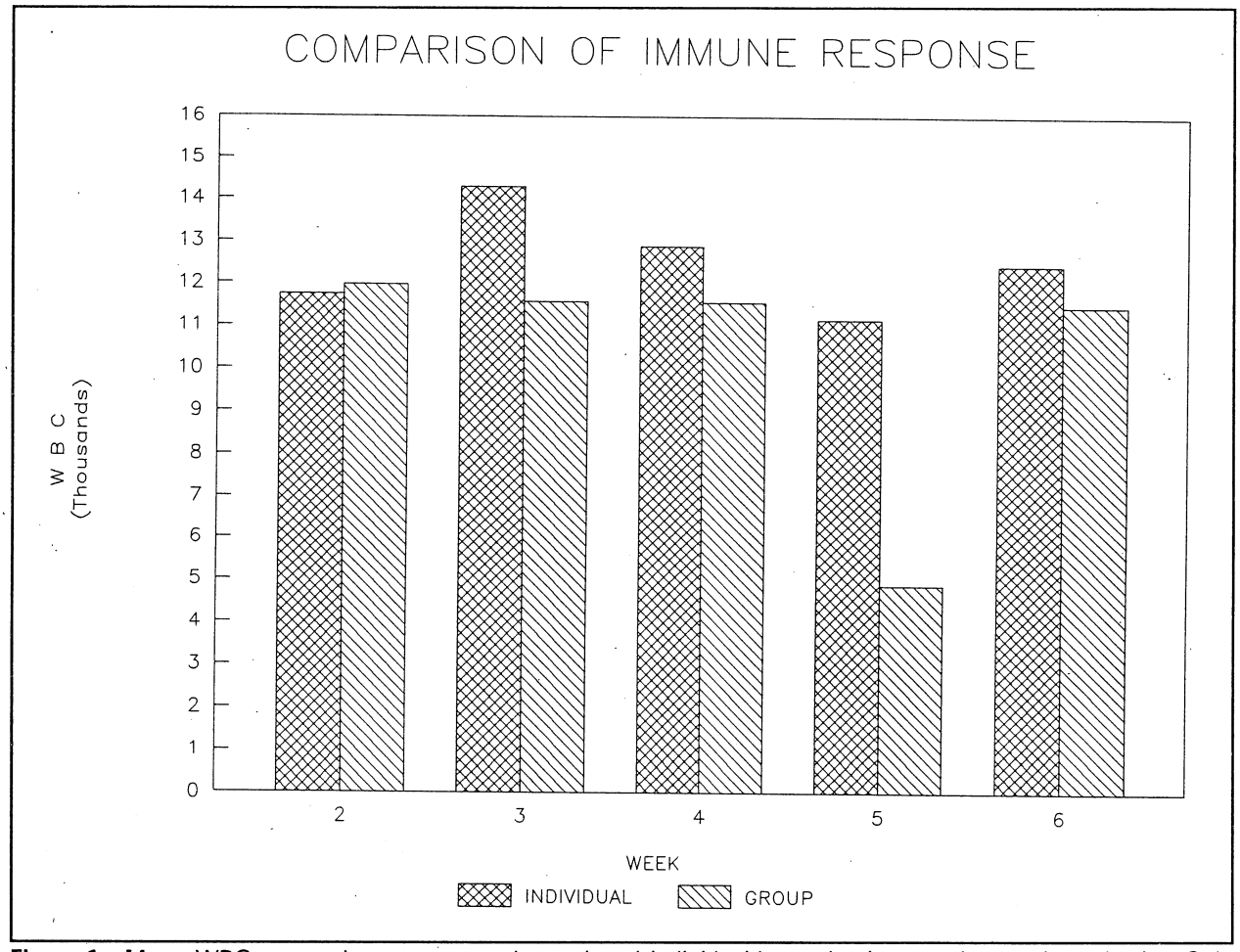

Figure 1. Mean WBC counts between group-housed and individual-housed mice, week two through six. Only significant difference is week five. Week one is not shown as technique was immature.

was to test whether or not a change in the housing condition would affect the immune system when challenged more than the state of the housing condition itself. One study showed that when animals were kept consistently in individual housing they had significantly higher antibody levels when injected with bovine serum than mice that were initially isolation housed then changed to group housing then placed again in isolation housing (Rabin et al, 1989). An abrupt change in social housing condition was shown to affect natural killer (NK) cytolytic responses. NK cell activity was increased in mice that were initially isolation housed then placed in group housing and decreased in mice that were initially group housed then placed in isolation housing (Hoffman-Goetz et al, 1991).

Materials and Methods

In this experiment, CD1 (Charles River Laboratories) mice were allowed to acclimate to a differentially housed condition for approximately three weeks prior to beginning of the study. Initially, twenty male mice were housed in isolation housing and were separated from twenty male mice that were group-housed, ten per cage. Regular grade Purina rat chow and tap water were provided ad libitum. Bedding was changed once per week at the time of drawing blood, and they were on a 12-hour light-dark schedule. Temperature varied from approximately 71 to 78°C.

To monitor the immune system, 20 microliters of blood were taken once per week from the suborbital sinus of the eye. This is considered the most efficient and least irritating method of obtaining blood (Green, 1966). Blood samples were diluted 1:9 with Turk's solution (97% H_2O, 3% CH_3COOH, 0.01% Crystal Violet). Total WBC counts were manually done using a hemocytometer at 400x magnification and recorded. Four days before blood was taken in the seventh week, each mouse was injected subcutaneously in the dorsal region [(0.06 ml/30 g mouse weight of chicken ovalbumin (1% ovalbumin in PBS, pH=7.2)]. At this

time, half of the initially individually-housed mice (n=10) were placed together in a group-housed condition and half of the initially group-housed mice (n=10) were placed in individual housing. The following day, all injections were repeated, and blood was taken three days later. Blood was taken again one week later before the termination of the experiment. The total WBC count data were first analyzed with MANOVA using SPSS/PC+ software. The counts from the sixth (pre-injection and pre-change in housing condition) and seventh (post-injection and post-change in housing condition) weeks were compared against the week (injection status), and the differential housing conditions.

Results

None of the results were significant (Table 1) except for the animals that ended up in group housing in the seventh week ($p=0.013$). These mice included those that were in individual housing in the first six weeks then made the change and those mice that were consistently in group housing. They had significantly higher WBC counts (increased immune response) than those mice that ended up in individual housing in the seventh week (Table 2).

Table 1. Results of MANOVA using nested comparisons.

Source of Variation	Significance of Differences
Within Cells	
Constant	.000
Final	.013*
Week	.467
Final by Week	.910

Table 2. Mean WBC counts in the seventh week (post-injection and post-change of housing condition) showing the difference between final housed in group or individual.

Housing Treatments	Cases	Mean WBC Counts
I*I	10	10,764 (3,891)
G*I	9	12,389 (5,513)
G*G	10	12,992 (3,575)
I*G	7	14,908 (4,119)

Discussion

Only male mice were used, since males have in general been shown to have stronger immune responses or more vulnerable immune systems. Changes in the immune system can therefore be noted with greater clarity. The CD1 strain was chosen as it has been used in differential housing studies.

The results of this study add to the evidence that variables affect differential housing studies in very different ways. Such variables that add to the divergence of results include type of stress challenging the immune system, duration of exposure to stress, sex, species, strain of animal involved, and presence of a hierarchial system in a group. The stress chosen for this study is a relatively moderate one of change in housing condition. These variables and the known variance of results precludes generalizations, and yet does assist in the further understanding of the mechanisms of the immune system. For example, studies have shown that differential housing affects glucocorticoid activity (Brain, 1975). This can be associated with an increased lymphocyte response as lymphocytes are known to have receptors for a variety of hormones including corticosteroids (one of the glucocorticoids that is produced by the adrenal cortex), insulin, catecholamines, growth hormone, and met-enkephalin (Roitt, et al., 1989).

The number of possible immune parameters that can be monitored include lymphocyte response, NK cell activity, organ weight, hormone titers, and antibody titers. In measuring immune response via WBC counts, much variance had to be expected. A summary of previous studies showing different strains of mice, mean and S.E. for total WBC count (all, female, and male), and the variance shown by S.E./mean indicated normal counts vary quite a bit (Green, 1966). Variance within strains ranged from a high of 35% to a low of 7%. The data from this study revealed an average variance among individuals within a sample period of 36%, while the average variance among sample periods for a single mouse was 32%. Results would be more clear with a lower variance, or another assay method should be used.

Given the large standard deviations of the means (Table 2), and the relatively small sample sizes of mice, it is possible to figure out how many animals must be in the sample to obtain significant results regarding the hypothesis. An estimate of sample size number necessary is roughly 10,000 mice using this data.

The mice were given weeks one through six to acclimate to the weekly blood drawing, and there were no significant differences in WBC count between the group-housed and isolation-housed animals except in week 5 (Figure 1). As nothing changed in this week with regards to treatment of animals, technique, or conditions, one explanation for the difference is that samples often sat in refrigeration for up to one week before being counted. Counts made within that week did not seem to be damaged or degraded. It is possible the particular samples from the group housed in week five degraded before being counted. Time may have been a factor.

Four days were allowed for WBC proliferation and release into the blood stream. This was not tested beforehand and determined using knowledge of WBC formation. It should also be noted that leukocytes, lymphocytes, and eosinophils have peak values in the morning, which are roughly three times the minimal values observed in the evening (Green, 1966). Blood was consistently drawn between 11:00 AM and 4:00 PM.

The group of animals that were initially housed in isolation (n = 9) then put together in a larger cage faced the additional stress of aggression among one another and probable territorial behavior. Mild fighting was noticed the first week they were together and when drawing blood the second time since their change, nicks and tears on their ears were noticed as well as scabs covering their tails and occasional wounds on their rumps. One mouse died from the wounds. During the time of drawing blood, one mouse was noted as not sleeping with the other mice and was considerably stronger. Identified as the dominant mouse, he was removed. Within the following week, the wounds healed and fighting was observed as nonexistent. The mice still gradually began to die from unknown complications.

The evidence from this study does not support the hypothesis that a change in housing condition affects the immune response in a more significant manner than when a change does not occur. However, if another stress, for example water-scheduling, were to be used in concordance with the housing condition change, and a more sensitive assay were used, the results may be more clear. The data does show evidence that group housing is more conducive to stimulation of the immune system.

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Acknowledgements

I would like to thank Dr. Donald "Buzz" Hoagland for his time and support regarding both the content and ethics of this project, and Dr. Jonathan Frye for his assistance in statistical analysis. Also, I would like to thank Erik Harmon for his generous time and dedication for this project.