MENTATION PAGE

Form Approved
OMB No. 0704-0188

2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
19 May 94	Final 1 June 93-31 May 94

4. TITLE AND SUBTITLE

Role of Cytokines in the Acute Phase Response

5. FUNDING NUMBERS

Grant #N00014-90-J-1547
RCT Code 341 7023

6. AUTHOR(S)

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n/a

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

Dept. of the Navy, Office of Naval Research
800 North Quincy
Arlington, VA 22217-5660

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT

DISTRIBUTION STATEMENT A
Approved for public release;
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13. ABSTRACT (Maximum 200 words)

We have characterized the physiological and behavioral responses to influenza infection in mice. Studies have been run showing that ip injection of antiserum to IL-1β increased survival rate in influenza-infected mice, and at the same time magnified the decrease of body temperature. We hypothesize that the antiserum inactivated biological activity of the large part of IL-1β, an endogenous pyrogen, thereby disturbing the ratio between pyrogenic and cryogenic input on thermoregulatory centers during influenza infection. This resulted in greater expression of the cryogenic input and exaggeration of the fall of body temperature. These data support our previous observation on mice infected with the virus that lowering of body temperature (i.e. anapyrexia) during influenza pneumonitis can confer a protective effect upon an organism.

We have also found that INFα and TNFα are antipyretic if given together with LPS into mice. Additionally, we observed an enhancement of the fever in mice injected with LPS and at the same time treated with TNF soluble receptor. These results confirmed our previous observation on mice treated with anti-TNFα serum and LPS, indicating that TNFα acts as the endogenous cryogen. We speculate that TNFα and INFα may account for anapyrexia in influenza-infected mice.

14. SUBJECT TERMS

Influenza, cytokines, sickness behavior, acute phase response
fever, anorexia

15. NUMBER OF PAGES

2

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
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FINAL PROGRESS REPORT

GRANT # N00014-90-J-1547 **R&T CODE: 3417023**

PRINCIPAL INVESTIGATOR: Matthew J. Kluger, Ph.D.

INSTITUTION: The Lovelace Institutes, Institute of Basic and Applied Medical Research

GRANT TITLE: **Role of Cytokines in the Acute Phase Response**

REPORTING PERIOD: 1/1/90-3/31/94

OBJECTIVE: The purpose of these studies is to assess in an animal model the roles of the cytokines IL-1, IL-6, TNF, and IFN in the changes in body temperature, reduction in activity, and anorexia/cachexia induced by acute infection.

APPROACH: Male specific pathogen free Swiss-Webster mice are inoculated with a mouse adapted strain of influenza (H1N1-A/PR/8/34) and activity, body temperature, food and water consumption, body weight, lung lavage fluid and plasma concentrations of cytokines are measured. Antiserum or control serum to specific cytokines (or other types of blockers of cytokine action) are injected to determine if blockade of cytokine action results in changes in the acute phase response.

ACCOMPLISHMENTS: We characterized the effects of various doses of influenza on body temperature, activity, food and water intake, and body weight in male mice. In addition, lung lavage fluid and plasma was assessed for IL-1, TNF, IL-6, and IFN. To evaluate a role of cytokines in pathophysiologic responses of mice during influenza pneumonitis, we tested the effects of the following cytokine antisera: anti-IFN and anti-IFN , anti-IL-6, anti-TNFα, anti-IL-1α and anti-IL-1ß, and a mixture (cocktail) of INF α+ ß antiserum and IL-6 antiserum, on changes in body temperature, motor activity and feeding behavior in mice inoculated with virus at a lethal dose of 55,000 PFU (10LD_{50}). We have not observed a significant effect of these antisera on measured parameters following this high dose of virus. In an ongoing study, we have found that when mice were infected with lower dose of influenza virus (140 PFU; 0.025 LD_{50}) and then treated with antiserum to IL-1ß survival rate increased in those mice and, at the same time, antiserum magnified the decrease of body temperature. We presume that in this experiment the antiserum inactivated biological activity of the large part of IL-1ß, an endogenous pyrogen, thereby disturbing a ratio between pyrogenic and cryogenic input on thermoregulatory centers during influenza infection. This resulted in greater expression of the cryogenic input and exaggeration of the fall of body temperature. These data support our previous observation on mice infected with the virus that lowering of body temperature (i.e. anapyrexia) during influenza pneumonitis can confer a protective effect upon an organism. We plan to do more experiments in this respect, applying a low dose of virus, poly I:C, and antibodies to various cytokines. We have found that INFα and TNFα are antipyretic if given together with LPS into mice. Additionally, we observed an enhancement of the fever in mice injected with LPS and at the same time treated with TNF soluble receptor. These results confirmed our previous observation on mice treated with anti-TNFα serum and LPS, indicating that TNFα acts as the endogenous cryogen. We speculate that TNFα and INFα may account for anapyrexia in influenza-infected mice. In separate experiments we have shown that hyperoxia (exposure of mice to 50% oxygen) partially prevented a drop of body temperature due to influenza virus. Hyperoxic conditions markedly shortened survival time of mice infected with the virus. We speculate that tissue hypoxia may develop in mice during influenza pneumonitis, and we that hypoxia may contribute to fall of body temperature. Since hyperoxic conditions are known to impose inhibitory effects on cyclooxygenase activity in the lungs, we tested whether anapyrexia in influenza-infected mice was prostaglandin-dependent, using indomethacin as a prostaglandin synthesis blocker. We observed that indomethacin also partially prevented the drop of body temperature induced by influenza. It did not, however, affect survival rate.

We have found that an ip injection of antiserum to IL-1ß increased survival rate in influenza-infected mice, and at the same time magnified the decrease of body temperature. We hypothesize that the antiserum inactivated biological activity of the large part of IL-1ß, an endogenous pyrogen, thereby disturbing the ratio between pyrogenic and cryogenic input on thermoregulatory centers during influenza infection. This resulted in greater expression of the cryogenic input and exaggeration of the fall of body temperature. These data support our previous observation on mice infected with the virus that lowering of body temperature (i.e. anapyrexia) during influenza pneumonitis can confer a protective effect upon an organism. We have also found that INF*α* and TNF*α* are antipyretic if given together with LPS into mice. Additionally, we observed an enhancement of the fever in mice injected with LPS and at the same time treated with TNF soluble receptor. These results confirmed our previous observation on mice treated with anti-TNF*α* serum and LPS, indicating that TNF*α* acts as the endogenous cryogen. We speculate that TNF*α* and INF*α* may account for anapyrexia in influenza-infected mice.

SIGNIFICANCE: Infections cause an array of "sickness" behaviors. Some infections cause a rise in body temperature and others a fall in temperature. The rise in temperature is known as fever, and the fall in temperature is "anapyrexia." The mouse provides an excellent model to study both fever (e.g. in response to injections of LPS) and anapyrexia (e.g. in response to infection with influenza virus or injection of poly I:C). Based in part on the support received from the ONR, we have initiated a broad series of investigations to study these phenomena, which focuse on the responses to influenza infection, which is a serious infection in military and civilian personnel.

PUBLICATIONS AND ABSTRACTS:

Majde, J.A., Dieffenbach, C., Havell, E.A., Kluger, M.J., and Maassab, H.F. Effects of tumor necrosis factor-*α* (TNF*α*) antibody on acute PR8 influenza in the mouse. VIII International Cong. Virology, Berlin, 411, 1990.

Kluger, M.J., Conn, C.A., McClellan, J.L., Maassab, H., Smitka, C., and Majde, J. TNF*α* levels, physiological responses and behavioral changes associated with influenza viral pneumonitis. Cytokine 3:510, 1991.

Klein, M.S., Conn, C.A., and Kluger, M.J. Behavioral thermoregulation in mice inoculated with influenza virus. Physiology and Behavior 52:1133-1139, 1992.

Kozak, W., Conn, C.A., and Kluger, M.J. Lipopolysaccharide induces fever and depresses locomotor activity in unrestrained mice. Am. J. Physiol., 266:R125-R135, 1994.

Conn, C.A., McClellan, J.L., Maassab, H.F., Smitka, C.W., Majde, J.A., and Kluger, M.J. Cytokines and the acute phase response to influenza virus in mice. Submitted for publication.

Kozak, W., Conn, C.A., and Kluger, M.J. Mechanism of anapyrexic thermoregulatory response to influenza pneumonitis in mice. I.U.P.S. Thermal Physiology Commission, A Symposium on Temperature Regulation, Aberdeen, Scotland, August, 1993.

It is anticipated that we will publish several additional papers based on the support received from ONR. One manuscript will involve our studies on the effects of neutralizing antiserum to IL-1ß and TNF*α* on the course of influenza infection. Another publication will be based on the characterization of poly I:C anapyrexia in mice (with a comparison to LPS-induced fever and influenza-induced anapyrexia). These experiments are close to completion, and manuscripts should be submitted by late summer, 1994.