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Prostaglandin E_2, Interleukin 4, Interleukin 10, and Transforming Growth Factor ß in Immunosuppression following endotoxemia. Accepted Journal of Trauma 1996.

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13. ABSTRACT *(Maximum 200 words)*

T-Cell suppressive factors (TSF) are thought to suppress host immunity and contribute to the development of sepsis. During the past year, we have investigated the relative roles of prostaglandin E_2 (PGE_2), Interleukin 4 (IL-4), Interleukin 10 (IL-10), and transforming growth factor $ß_1$ ($TGFß_1$) as immunosuppressive factors in our rabbit endotoxemia model. Endotoxemia suppresses *in vivo* cell mediated immune function and increased PGE_2, IL-4, IL-10, and $TGFß_1$ levels are measurable. Serum from these animals following endotoxemia suppresses T-cell proliferation and IL-4, IL-10, $TGFß_1$, and PGE_2 had TSF activities of 530, 102, 12, and .37 U/ng. $TGFß_1$, IL-10, and IL-4, contributed 37, 32, and 14 U/ml to a total serum TSF activity of 614 U/ml, while PGE_2 contributed only .007 U/ml. These results show that $TGFß_1$, IL-10, IL-4, and other uncharacterized factors, are potent T-cell suppressors following endotoxemia in rabbits. PGE_2 is of much less significance.

14. SUBJECT TERMS

Endotoxemia, immunosuppression, IL-4, IL-10, PGE_2, $TGFß_1$.

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ANNUAL PROGRESS REPORT

Grant #: N00014-91-J-1723 **R&T Code:** 4123008

PRINCIPAL INVESTIGATOR: David B. Hoyt, M.D.

INSTITUTION: University of California, San Diego

GRANT TITLE: Evaluation of the Mechanism of Immunosuppression and Calcium Homeostasis by an Immunosuppressive Trauma Peptide

REPORTING PERIOD: 1 June 1994 - 31 May 1995 (12 months)

AWARD PERIOD: November 1993 - November 1995

OBJECTIVE: To purify and characterize T-cell suppressive factors occurring after trauma and sepsis.

APPROACH: Our interest in purifying T-cell suppressive factors (TSF) from injured septic patient serum has confirmed the presence of suppressive factors and the involvement of calcium signaling as the mechanism of action. Because of the difficulty in isolating an individual suppressive factor and the knowledge that multiple factors contribute to suppression, we have explored the roles of PGE_2, IL-4, IL-10 and TGF_β on immunosuppression following endotoxemia. We have further evaluated the effect of blocking TNF following sepsis on TSF. The importance of Na^+ concentration and the potential benefit of this on resuscitation of T-cell function was realized during peptide isolation. Because of this, we have explored the effect of hypertonic saline (HTS) on T-cell suppression.

ACCOMPLISHMENTS (last 12 months):

Roles of PGE_2, IL-4, IL-10, and TGF β following endotoxemia:
The relative roles of PGE_2, IL-4, IL-10, and $TGF\beta_1$ as immunosuppressive factors in a rabbit endotoxemia model were tested. Endotoxemia suppressed *in vivo* cell mediated immune function and increased plasma PGE_2, IL-4, IL-10, and $TGF\beta_1$ levels. Serum after endotoxemia suppressed T-cell proliferation of normal rabbits. IL-4, IL-10, $TGF\beta_1$, and PGE_2 had TSF activities of 530, 102, 12, and .37 U/ng. $TGF\beta_1$, IL-10, and IL-4 contributed 37, 32, and 14 U/ml of the total serum TSF activity of 614 U/ml, while PGE_2 contributed only .007 U/ml. $TGF\beta_1$, IL-10, IL-4, and other uncharacterized factors are the primary T-cell suppressors following endotoxemia in rabbits. (1)

Effect of anti-TNF antibody treatment on production of TSF in septic baboons:
We have also examined whether tumor necrosis factor (TNF-a) antibody treatment influences the production of TSFs, including IL-10 and TGF-β. Sepsis induced in baboons by *E. coli* infusion caused an increase in plasma levels of TNF, TSF activity, IL-10, and active TGF-β. TNF antibody pre-treatment reduced TNF levels by 98%. Transient TSF activity (0-4h) was only marginally influenced, while sustained TSF activity (8-24h) was markedly reduced. (2)

The effect of HTS on PGE_2 induced in vitro T-cell suppression:
The action of increased concentrations of HTS, hypertonic saline - Dextran (HSD), Dextran (Dx), albumin (ALB), and Hetastarch (HET) on in vitro proliferation of phytoheamgglutinin-stimulated (PHA) normal and PGE_2-suppressed human peripheral blood mononuclear cells was tested. At clinically relevant levels, HTS, HSD (20-40 mM hypertonicity), ad ALB (2.5 mg/ml)enhanced T-cell proliferation by 65%, 75%, and 70%, respectively. Dx and HET had little effect. HTS also reversed PGE2-suppressed (10 ng/ml) T-cell proliferation to normal levels, and HSD enhanced T-cell proliferation by 40%. (3)

HTS enhances splenocyte proliferation & IL-2 but blocks IL-1, IL-6 and TNF:
Splenocytes from healthy BALB/c mice were cultured in the presence of increasing concentrations of NaCl

(10 mM to 80 mM above isotonicity) for 24 hours. T-cell proliferation and IL-2 levels were measured following PHA stimulation. Production of the monokines TNFa, IL-1β and IL-6 was induced by LPS. Na^+ concentrations ranging from 20 mM to 40 mM above isotonicity enhanced T-cell proliferation and IL-2 production, up to 150 %. TNFa, IL-1β and IL-6 production was significantly reduced at increasing HTS concentrations. (4)

The effect of HTS on in vivo immune cell function:
BALB/c mice were injected with 7.5% HTS (24 ml/kg intraperitoneally) increasing plasma Na^+ to levels seen in HTS resuscitated trauma patients. *In vivo* immune function was assessed by delayed-type hypersensitivity (DTH) testing. Serum IL-1, IL-2, IL-6, and the effect of serum on *in vitro* T-cell proliferation were measured after 6 and 24 h. *In vivo* DTH, the effect of serum on *in vitro* T-cell proliferation, and IL-6 production were significantly increased in HTS animals. While IL-2 levels in the HTS group were twice as high as those in the control group, they did not reach statistical significance as predicted from the *in vitro* experiments. (5)

The effect of HTS resuscitation on hemorrhage induced immunosuppression:
Hemorrhage was induced in BALB/c mice by bleeding to a mean arterial blood pressure of 35±10 mmHg for 60 minutes. PHA induced splenocyte proliferation and the plasma levels of IL-1, IL-2, IL-4, IL-10 and TGF-β were measured. *In vivo* cell mediated immune function was measured by DTH. Hemorrhage induced suppression of splenocyte proliferation was prevented with HTS resuscitation. *In vivo* cell mediated immune function was significantly improved by HTS. HTS resuscitated animals showed lower levels of antiinflamatory cytokines IL-4, IL-10 and TGF-β and higher levels of the proinflamatory cytokines IL-1 and IL-2 compared to the Ringer's lactate resuscitated control group. (6)

SIGNIFICANCE:
Circulating suppressive substances following injury, systemic inflammation, or gram-negative sepsis contribute to post injury immune suppression and infection related morbidity. The ability to modulate these suppressive factors, could effect post-traumatic morbidity and mortality.

WORK PLAN (NEXT 12 MONTHS):
Characterization of cellular mechanisms of IL-4, IL-10, and TGFβ leading to T-cell suppression.
Contribution of uncharacterized TSFs on T-cell proliferation.

PUBLICATIONS AND ABSTRACTS (last 12 months):
1. Junger WG, Hoyt DB, Liu FC, Loomis WH. Immunosuppression following endotoxin shock - the result of multiple anti-inflammatory factors. Abstract- The American Association for the Surgery of Trauma, Halifax, Canada, J. Trauma (in press), 1995

2. Junger WG, Hoyt DB, Redl H, Liu FC, Loomis WH, Davies J, Schlag G. Tumor necrosis factor antibody treatment of septic baboons reduces the production of sustained T-cell suppressive factors. Shock 3:173-178, 1995.

3. Coimbra R, Junger WG, Liu FC, Loomis WH, Hoyt DB. Hypertonic/hyperoncotic solutions reverse PGE2-induced T-cell proliferation in vitro. Shock 4(1):44-49, 1995.

4. Coimbra R, Junger WG, Liu FC, Loomis WH, Hoyt DB. Hypertonic saline enhances T-cell proliferation and IL-2 production but blocks IL-1, IL-6, and TNF production. Abstract- The American Association for the Surgery of Trauma, Halifax, Canada, J. Trauma (in press), 1995.

5. Coimbra R, Junger W, Hoyt D, Liu F, Loomis W. Hypertonic saline augments T-cell IL-2 and IL-6 production. Shock 3 (suppl), 1995.

6. Coimbra R, Junger WG, Hoyt DB, Liu FC, Loomis WH, Evers MF, Davis RE. Immunosuppression Following Hemorrhage is Reduced by Hypertonic Saline Resuscitation. Surgical Forum (in press), 1995.

FORM 2--ANNUAL REPORT QUESTIONNAIRE, CALENDAR YEAR 1995
(for ONR use only)

Principal Investigator Name: David B. Hoyt, M.D.

Institution: University of California, San Diego

Project Title: Evaluation of the Mechanism of Immunosuppression and Calcium Homeostasis by an Immunosuppressive Trauma Peptide

Number of ONR supported

Papers published in refereed journals: 3

Papers or reports in non-refereed publications: 0

Books or book chapters published: 1

Number of ONR supported inventions/patents or licensed technologies:

Disclosed: 0

Filed: 0

Granted: 0 Patent No(s):

(describe in detail on Form 1)

Number of seminars/presentations

Invited: 5

Contributed: 3

Trainee Data (for those receiving full or partial ONR support):

	TOTAL	FEMALE	MINORITY	NON-US CITIZEN
No. Grad. Students:	0	-	-	-
No. Postdoctorals:	2	-	-	2
No. Undergraduates:	2	-	-	-

AWARDS/HONORS TO PI AND/OR TO MEMBERS OF PI'S RESEARCH GROUP (please describe):

American College of Surgeons Committee on Trauma Region IX Basic Science Research Award, 1995
Chancellor's Associates Faculty Award for Outstanding Community Service,1995

No. of animals used; each species: 60 Rabbits 160 Mice

Equipment purchased on grant (number and description of items costing >$1,500): None

David B. Hoyt, M.D. **May 1995**

PROJECT HIGHLIGHTS

Prostaglandin E_2, Interleukin 4, Interleukin 10, and Transforming Growth Factor β in Immunosuppression following endotoxemia.

T-cell suppressive factors (TSF) are thought to suppress host immunity and contribute to the development of sepsis. During the past year, we have investigated the relative roles of prostaglandin E_2 (PGE_2), Interleukin 4 (IL-4), Interleukin 10 (IL-10), and transforming growth factor β_1 ($TGF\beta_1$) as immunosuppressive factors in our rabbit endotoxemia model.

Endotoxemia suppressed *in vivo* cell mediated immune function and increased plasma PGE_2, IL-4, IL-10, and $TGF\beta_1$ levels (Figures 1, 2, 3, 4, 5, 6, 7, 8 - Appendix).

Serum after endotoxemia suppressed T-cell proliferation of normal rabbits. IL-4, IL-10, $TGF\beta_1$, and PGE_2 had TSF activities of 530, 102, 12, and .37 U/ng. $TGF\beta_1$, IL-10, and IL-4 contributed 37, 32, and 14 U/ml to a total serum TSF activity of 614 U/ml, while PGE_2 contributed only .007 U/ml (Table 1 - Appendix).

These results show that $TGF\beta_1$, IL-10, IL-4, and other uncharacterized factors, are potent T-cell suppressors following endotoxemia in rabbits. PGE_2 is of much less significance.

We plan to investigate the relative effects of these factors on intracellular T-cell calcium signaling and antigen expression to explore their effect on these known pathophysiological effects of trauma and injury.

MAJOR PROBLEMS:

There have not been any major problems during the last year, however,the complexity of immune suppression and the contribution of multiple factors made the likelihood of isolating a single factor less plausible.

POTENTIAL PATENTABLE INVENTIONS:

None