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Microenvironmental Control of Monokine Production in Wound Repair

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13. ABSTRACT *(Maximum 200 words)*

The macrophage is an important regulatory cell in host defense and wound healing. The ability of the macrophage to respond to various microenvironmental stimuli may be an important regulatory mechanism. Using a constant perifusion tissue culture system and human peripheral blood monocytes, we rigidly controlled oxygen environments, pH and CO2. Hypoxic and normoxic oxygen environments (2% and 20%) were tested with and without gamma interferon. The collected, concentrated supernatants were then tested for PDGF, TGFB, TNF, IL-1 alpha and beta, IL-6, and angiogenesis activity. Hypoxia increased production of angiogenesis. Levels of TGFB and nitrous oxides were present and similar in both oxygen environments, while PDGF and IL-1 were not detected. The level of IL-6 was decreased in hypoxia and the combination of hypoxia and IFN drives IL-6 production to zero. Further experimentation on culture conditions showed that there was continued macrophage death during the perifusion and scanning electron micrographs showed that the plating density was lower than anticipated. These experiments are the first attempt to our knowledge to use constant perifusion of macrophages to model the microenvironments these cells are exposed to in vitro.

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Final Report

GRANT #: N00014-90-J-1895 R&T
CODE: Ser 1141SBS/134/JAM

PRINCIPAL INVESTIGATOR: David R. Knighton, MD

INSTITUTION: University of Minnesota

GRANT TITLE: Microenvironmental Control of Monokine Production in Wound Repair

AWARD PERIOD: 1 June 1990-31 May 1993

OBJECTIVE: To investigate the effect of the wound space oxygen, lactate, and pH microenvironment on the regulation of monokine production and to determine the interrelationship between these different environmental conditions and known biochemical modulators of macrophage function on monokine production.

APPROACH: Utilizing a computer-controlled, constant perfusion, tissue culture system (Opticell) which allows for precise control of the cellular environment, we determined the effect of various oxygen environments and biochemical macrophage modulators on macrophage production of platelet derived growth factor (PDGF), transforming growth factor ß (TGFß), tumor necrosis factor alpha (TNFa), and macrophage derived angiogenesis factor (MDAF). Standard assays for these growth factors were used including ELISA and cellular assays for PDGF, TGFß, TNFa, IL-1 alpha and beta and IL-6. MDAF was measured using the qualitative rabbit corneal implant assay.

ACCOMPLISHMENTS: Our accomplishments during this grant period can be divided into four sections:
1: Refinement of the system to culture and maintain human peripheral blood monocytes/ macrophages in a constant flow tissue culture system. We had extensive expertise in culturing all types of macrophages in static culture. When we attempted to transfer this knowledge to the Opticell, constant flow tissue culture system, we had to basically start from scratch. As our knowledge of the system increased and after we switched to a serum-free media (HL-1) instead of human serum in the culture media, we were finally able to conduct the proposed experiments.

2: We then used the Opticell system to reproduce our previously published data on the environmental control of macrophage derived angiogenesis factor production. The Opticell system provided the same result that we determined in static culture. Air environments containing 20% O_2 shut

down angiogenesis factor production while hypoxic conditions (2% O_2) induce angiogenesis factor production. We then completed some cycle experiments which showed that the angiogenesis factor production was turned off when the same set of macrophages were exposed again to hyperoxic conditions. Since we had successfully repeated our earlier experiments we proceeded with experiments on biochemical regulation of monokine production.

3: Using gamma interferon (IFN) as a monocyte modulator, we studied the production of PDGF, TNFa, TGFß, IL-1 and IL-6 at both normoxic and hypoxic oxygen environments. We found that TNFa production remained relatively constant under normoxia and hypoxia but was significantly reduced in the presence of IFN. No detectable amounts of PDGF, IL-1 alpha or IL-1ß were measured in any of the supernatants. Macrophage angiogenesis activity was affected by IFN. IFN reduced the amount of angiogenesis from hypoxic macrophages and had no effect on the normoxic cells. From these experiments we concluded that hypoxia induced production of MDAF and that this production was decreased in the presence of IFN. The production of IL-6 was reduced under hypoxic conditions and in the presence of IFN. The combination of IFN and a hypoxic environment reduced IL-6 production to nearly zero.

4. A critique of this data which was presented at the Surgical Infection Society prompted us to try and determine the number of cells which adhered to the core of the Opticell and to determine their viability over time. We felt that this was an important parameter to address, so we measured LDH in the conditioned media over time and found that there was an increase in the LDH and therefore cell death over time. In an attempt to look at the macrophages in the core, we then seeded the core and did scanning electron micrographs at various times. We found that monocytes adhered to the core and spread out in a manner similar to static culture. To our surprise, the number of cells per channel was low. At the end of the project we were attempting to seed the cores with rabbit bone marrow macrophages in large numbers to test whether their human counterparts from leftover bone marrow transplantation could be used instead of peripheral blood monocytes.

CONCLUSIONS: Human peripheral blood monocytes can be cultured in constant perfusion culture using a serum-free media (HL-1) instead of human serum. These cells produce monokines and respond to changes in their oxygen tension to induce angiogenesis factor production under hypoxia. IFN decreased angiogenesis factor and IL-6 production under normoxic and hypoxic conditions. IFN inhibits TNF production only under hypoxic conditions. The seeding density of peripheral blood monocytes in the Opticell core was low and there was early evidence of ongoing cell death.

SIGNIFICANCE: These experiments provide important data on the culture of macrophages and monocytes in a novel constant perfusion system which can be manipulated to more closely model the microenvironment found in living tissue than can be achieved utilizing standard static culture conditions. Low oxygen tensions such as those found in healing wounds and infections induce angiogenesis while IFN is an important immune modulator. In concert, these two factors may play an important role in the regulation of monokine production during the wound healing response.

PATENT INFORMATION: There were no patents submitted from this work.

PUBLICATIONS AND ABSTRACTS:

Knighton, D.R., Atkinson, K.L., and Fiegel, V.D. Environmental modulation of monokine production in a constant perifusion system. Presented at the Twelfth Annual Meeting of the Surgical Infection Society. April, 1992.

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PROGRAM

TWELFTH ANNUAL MEETING

Thursday, April 09 through Saturday, April 11

1992

Headquarters:

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LOS ANGELES, CALIFORNIA

19. ENVIRONMENTAL MODULATION OF MONOKINE PRODUCTION IN A CONSTANT PERIFUSION SYSTEM

DR Knighton, MD
KL Atkinson, BS
VD Fiegel, BS
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Discussant: Frank Cerra

The macrophage is an important regulatory cell in a variety of biologic processes including host defense, septic shock, and wound healing. The ability of the macrophage to respond to various microenvironmental stimuli such as pO_2, pH, metabolic byproducts, and locally acting biochemical mediators is an important mechanism which allows control of macrophage function. The current studies were designed to examine the effect of pO_2 and γ-interferon (γIFN) on unstimulated human peripheral blood monocyte (PBM) growth factor production. PBM were obtained by leukopheresis and isolation on a Ficoll/Hypaque gradient. The PBM were cultured using an automated cell culturing system which provides continuous perifusion of the cells and rigidly maintains the culture conditions including pO_2 and pH. PBM were cultured in a serum-free media (HL-1, Ventrex Labs) under normoxic (20% O_2) conditions for seven days prior to the start of the studies. Environmental parameters to be tested were normoxic and hypoxic (2% O_2) conditions with or without 300 U/ml γIFN. The PBM conditioned supernatants were collected after 24 hours at each environmental condition, dialyzed, concentrated 30-fold, and analyzed by EIA for the presence of tumor necrosis factor-α (TNFα), interleukin-6 (IL-6), platelet-derived growth factor (PDGF), interleukin-1α (IL-1α), and interleukin-1β (IL-1β). The results from 3 studies are shown below, with data presented as % of control utilizing the normoxic conditions as the control values.

Culture Conditions	TNFα (% of control)	IL-6 (% of control)
20% O_2	100	100
2% O_2	96	56 *
γIFN + 20% O_2	35	18 *
γIFN + 2% O_2	26 *	4 *

* $p<0.05$

No detectable levels of PDGF, IL-1α, or IL-1β were found. These results indicate that hypoxia does not effect TNFα production while γIFN treatment alone greatly reduces both TNFα and IL-6 production. Hypoxia alone significantly decreases IL-6 production, as does γIFN alone, and the combination of hypoxia and γIFN drives IL-6 production to essentially zero. These results provide further evidence for the importance of the microenvironment in the regulation of macrophage function.