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Anti-lymphoproliferative activity of alpha-2-macroglobulin in the plasma of hibernating 13-lined ground squirrels and woodchucks

Donna G. Sieckmann[a,*], Howard Jaffe[b], Susanne Golech[a], DeCheng Cai[c], John M. Hallenbeck[c], Richard M. McCarron[a,d,*]

[a] Naval Medical Research Center, Silver Spring, MD 20910, United States
[b] LNC-NINDS Protein/Peptide Sequencing Facility, NINDS, NIH, Bethesda, MD 20892, United States
[c] Stroke Branch, NINDS, NIH, Bethesda, MD 20892, United States
[d] Department of Pathology, USUHS, Bethesda, MD 20814, United States

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ABSTRACT

Plasma from hibernating (HIB) woodchucks (*Marmota monax*) or 13-lined ground squirrels (*Ictidomys tridecemlineatus*) suppressed ^{3}H-thymidine uptake in mouse spleen cell cultures stimulated with Concanavalin A (ConA); plasma from non-hibernating animals were only slightly inhibitory. Maximum inhibition occurred when HIB plasma was added to the cultures prior to ConA. After HPLC size exclusion chromatography of the HIB ground squirrel plasma, a single fraction (fraction-14) demonstrated inhibitory activity. Assay of fraction-14 from 8 HIB squirrels showed inhibition ranging from 13 to 95%; inhibition was correlated to the time the squirrels were exposed to cold prior to hibernation. Western blot analysis showed the factor to be a large molecular weight protein (>300 kDa), and mass spectrometry identified sequences that were 100% homologous with alpha-2-macroglobulin from humans and other species. These findings indicate a hibernation-related protein that may be responsible for immune system down regulation.

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1. Introduction

Hibernation is a unique biological adaptation, in which an animal lowers its body temperature as well as its metabolic-, heart-, and respiratory-rates (Boyer, 1999), in order to allow survival through extreme temperatures and environmental conditions. Multiple cellular and molecular changes are probably required to bring about these metabolic changes. In keeping with the requirement to lower energy expenditure, a natural means of immunosuppression is in operation during hibernation (Bouma et al., 2010). In hibernating frogs, depletion of lymphocytes and hematopoietic cells from the blood, marrow, and lymphatic organs leads to a decreased plaque-forming cell response (Cooper et al., 1992). Hibernating hamster spleen fragments immunized to sheep red blood cells in vitro have a lower antibody response than fragments from non-hibernating hamsters (Sidky and Auerbach,

Abbreviations: CA, cold-adapted; ConA, concanavalin A; HIB, hibernating; SA, summer-active; SEC, size exclusion chromatography; WAX, weak anion exchange chromatography; WA, winter-active.

* Corresponding authors at: Neurotrauma Department, Naval Medical Research Center, 503 Robert Grant Avenue, Silver Spring, MD 20910-7500; Tel.:+1 301 319 7474.
E-mail addresses: donna.sieckmann@med.navy.mil, sieckmann@erols.com (D.G. Sieckmann), richard.mccarron@med.navy.mil (R.M. McCarron).

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1968). Hibernating animals have been reported to be unable to respond to vaccination before (Jaroslow and Smith, 1964) or during (Andjus and Matic, 1959) a bout of hibernation. During hibernation, the mitotic activity of cells and tissues has been shown to be dramatically decreased: mitoses are absent or are displayed as atypical forms (Kolaeva et al., 1980). Flow-cytometry studies have shown that during the entire hibernation bout, there is an accumulation of cells of various organs in the G_1 phase (Kolaeva et al., 1980). Other studies have shown that in the tissues of a hibernating animal, there is a decrease in DNA, RNA, and protein synthesis (Kolaeva et al., 1980). DNA synthesis is suppressed in hibernating hamster tissues with high mitotic activity, such as lymphoid tissue (Manasek et al., 1965). Hibernation-related suppression of cellular proliferation has even been shown to negatively affect the growth tumor cells transplanted into the cheek pouch of hibernating hamsters (Lyman and Fawcett, 1954). Thus, in addition to the depletion of lymphocytes, immunosuppression may be related to a general suppression of cellular proliferation in various hibernating species.

Increasing evidence suggests that factor(s) controlling metabolic changes during hibernation might be circulating in the blood and/or present in other body fluids. Originally it was reported that plasma from a hibernating ground squirrel could induce hibernation when transferred to a summer active ground squirrel (Dawe and Spurrier, 1969). In other experiments, an albumin-containing fraction derived from hibernating squirrel plasma was shown to induce hypothermia, bradycardia, and lethargy, when injected intracerebrally into rhesus monkeys (Myers et al., 1981). Albumin containing fractions of hibernating woodchuck plasma also inhibit growth of nutrient-starved tissue cultured cells (Chien and Oeltgen, 1993). Other investigators demonstrated that acetone extracts of brain tissue from 13-lined ground squirrels decreased the thymidine uptake by Chinese hamster ovary cells in culture (Amorese et al., 1982). Non-protein extracts from intestinal tissue of hibernating ground squirrels induced body temperature depression and decreased oxygen consumption in mice (Kramarova et al., 1993); these extracts also inhibited development of sea urchin embryos. Similarly, a small molecular weight fraction of an extract of brown fat from hibernating ground squirrels suppressed the proliferation of mitogen stimulated mouse lymph node cells (Atanassov et al., 1995).

Because of these findings and observations that plasma from a hibernating ground squirrel could induce a hibernating state (Dawe and Spurrier, 1969), we investigated the possibility that plasma from hibernating animals might have circulating factors which suppress lymphocytic function. An assay of mitogen-induced T-lymphocyte proliferation was used to discover an inhibitory factor in the plasma from hibernating 13-lined ground squirrels and woodchucks (Sieckmann et al., 2004). Purification of this "factor" shows that the inhibitory activity is associated with a large molecular weight protein (native >300 kDa, reduced 175 kDa), which identifies with sequences in the alpha-2-macroglobulin family of proteins from humans and other species.

2. Materials and methods

2.1. Animals and plasma samples

2.1.1. Ground squirrels and ground squirrel plasma

Thirteen-lined ground squirrels (*Ictidomys tridecemlineatus*), trapped in the late summer, were obtained in late August from a United States Department of Agriculture-licensed trapper (TLS Research, Chicago, IL). They were housed individually in a conventional animal room with an ambient room temperature of 21 °C and a 12:12-h light-dark cycle, and fed rodent chow and water ad libitum. Beginning late October, animals which showed increased body weight (average wt. 207 ± 24 g) were housed separately in cages containing wood shavings, supplied with food and water ad libitum, in an environmental chamber (hibernaculum) which was maintained in constant darkness, except for a red safe light (3–5 lx), at 5–6 °C, and 60% humidity, to induce torpor. Wood shavings were placed on the dorsal surface of animals which had burrowed into the bedding; the animals were inspected daily to assess the state of hibernation or arousal. Heparinized blood samples were taken between January and April by cardiac puncture from squirrels that had been assessed as hibernating (no movement of wood shavings) for at least 48 h (average rectal temperature of 7.5 ± 3.0 °C). Other animals which kept a low and stable body weight (average wt. 174 ± 16.9 g) remained in the conventional animal room (i.e. non-hibernating) and were designated "winter-active" (WA). Animals that were in the hibernaculum for 8–20 days, but showed no signs of hibernation, were referred to as cold-adapted active animals (CA). Blood samples were collected by cardiac puncture from WA squirrels (average rectal temperature of 37.2 ± 1.1 °C) and CA squirrels (average rectal temperature of 36.5 ± 0.8 °C) under anesthesia with ketamine (75 mg/kg, intraperitoneally). Blood samples were also obtained from squirrels after they arrived from the vendor, the first week of September; these squirrels are referred to as summer-active (SA). Individual squirrel blood samples were centrifuged at $1200 \times g$ to separate the plasma fraction, which was stored in 100–500 μl aliquots at −80 °C. Experiments tested either individual squirrel plasma samples or mixtures of plasma pooled from eight squirrels for HIB, CA, and WA, and four squirrels for SA. Plasma pools were aliquoited and stored at −80 °C until use in experiments. The experimental procedures were approved by the National Institute of Neurological Disorders and Stroke Animal Care and Use Committee.

2.1.2. Woodchuck and rat plasma

Woodchuck plasma samples were purchased from Northeastern Wildlife, Plymouth, NY. The plasma samples were derived from individual woodchucks (*Marmota monax*), trapped in the upper New York state area, that either 1) had been hibernating in captivity for 30 days (HIB plasma, from two animals, collected in December and February) or 2) was from a non-hibernating, summer-active animal (SA plasma, collected from two animals in May and September). Heparinized rat plasma was purchased from PEL Freez Biologicals, Rogers, AR. These

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