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AGE-RELATED DIFFERENCES IN THE INDUCTION OF 2-5A SYNTHETASE AND 2-5A DEPENDENT BINDING PROTEIN ACTIVITIES BY INTERFERON IN GUINEA PIG PERITONEAL MACROPHAGES

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2-5A synthetase and binding protein activities in peritoneal macrophages have been compared between young (6 month) and old (22-24 month) guinea pigs. Enzyme activities are lower in aged animals with a 17% and a 31% reduction in synthetase and binding protein activities, respectively. In addition, the response to the addition of mouse fibroblast interferon by macrophages from these two age groups is also substantially different. Whereas addition of interferon to young guinea pig macrophages elicits a 3.8- and a 1.7-fold increase in the synthetase and binding protein activities, only a marginal elevation in these two enzyme activities is found with interferon-treated old guinea pig macrophages. Analysis by thin layer chromatography demonstrates a marked difference in the relative distribution of the various oligomeric forms of 2-5A synthesized by young or old guinea pig macrophages. The binding protein in old animals appears to be significantly more thermolabile than the corresponding activity from young animals. The altered response to interferon and the difference in enzymatic properties in aged animals may represent part of the mechanisms involved in the progressive loss of the adaptative ability of an organism to environmental changes during senescence.

The process of aging is associated with a gradual reduction in the adaptative ability of an organism to environmental changes. Such a decrease is often manifested at the biochemical level by a decline in the capacity of some macromolecules to adjust to challenges imposed by the addition of hormones, diets, or pharmacological agents. For example, old age in humans is accompanied by the increased proneness to severe viral infections (1,2). This age-related enhanced viral susceptibility probably involves multiple causes and most likely includes a reduced potential of aged cells to interact with antiviral agents and other biological modifiers (1-6). Because macrophages are considered to be of central importance in immunosurveillance and in host resistance to bacteria, fungi, viruses and tumor cells (7-10), we have investigated the interaction between the

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established antiviral proteins, interferons, and peritoneal macrophages derived from young and old guinea pigs. We have concentrated on 2-5A synthetase and 2-5A dependent binding protein since these two enzymes have been implicated in the establishment of the antiviral state in murine and human cells (11-18). Our results show that marked differences exist in young and aged macrophages in their response to the addition of interferon. A preliminary report has appeared (19).

MATERIALS AND METHODS

Maintenance of guinea pigs, induction of peritoneal macrophages by mineral oil injection, isolation of macrophages and preparation of their cell free extracts have been described in a previous publication (20).

2-5A synthetase and 2-5A binding protein activities were assayed and characterized as previously described (20-23).

RESULTS AND DISCUSSION

Ageing is known to be associated with a reduction in cellular immune responsiveness (1-5). Previous investigations on the age-related decline of immune competence are mostly directed toward the functional expression of T and B cells. Thus, advancing age is accompanied by a decrease in lymphocyte response to mitogens and antigens, and in cellular cytotoxicity (1,4,5). There have been reports on the reduced capacity of aged immune cells to synthesize cytokines (3). Because macrophages are among the key primary cells in modulating the cell-mediated immune response (7-10), we have compared the interaction of macrophages derived from young and old guinea pigs with interferon.

Induction of 2-5A Synthetase and 2-5A Binding Protein by Mouse Fibroblast Interferon in Young and Old Guinea Pig Macrophages. In a previous study, we have established the cross species interaction between mouse fibroblast interferon and guinea pig cells by (i) demonstrating the antiviral state in interferon-treated guinea pig macrophages, (ii) morphological studies, (iii) measurement of tumor cytostatic activity, and (iv) the induction of 2-5A synthetase and 2-5A dependent endoribonuclease (20,24). Table 1 compares the induction of these two enzymes by mouse interferon in young and old macrophages. While the 2-5A synthetase activity is induced 3.8-

Table 1
A Comparison of 2-5A Synthetase and 2-5A Binding Protein Activities in Young (6 month) and Old (22-24 month) Guinea Pig Peritoneal Macrophages

Age	2-5A Synthetase (nmole/mg/h)		2-5A Binding Protein (fmole/mg/h)	
	-IFN	+IFN[a]	-IFN	+IFN
6 month	9.7	48	174	556
	7.0	16	189	547
	8.3	15	334	498
	5.2	32	325	357
	4.8	23	205	235
			145	492
			348	298
Mean±S.D:	7.0±2.1 (1)	26.8±13.7 (3.8)[b]	245±87 (1)	426±128 (1.7)[c]
22-24 month	7.0	8.0	65	64
	6.0	7.7	292	348
	4.2	7.1	400	370
	5.0	6.4	139	149
	6.8	6.2	93	125
			96	120
			106	122
Mean±S.D:	5.8±1.2 (1)	7.1±0.79 (1.2)	70±126 (1)	185±121 (1.1)

[a] Mouse, fibroblast interferon (50 units/ml) was incubated with macrophages for 17h.
[b] Significant at p=0.0125
[c] Significant at p=0.0093

fold in young macrophages, it is elevated only 1.2-fold in old macrophages. Similarly, there is a 1.7-fold increase in the 2-5A binding protein activity in interferon-treated young macrophages and only a marginal increase in the corresponding old macrophages. Mixing experiments using extracts from young and old macrophages does not indicate the presence of 'factor(s)' which may stimulate or inhibit these two enzyme activities. Another associated characteristic of aged guinea pig macrophages is the 17% and the 31% decrease in 2-5A synthetase and 2-5A binding protein activities (Table 1).

Relative Distribution of Various forms of Enzymatically Synthesized 2-5A. 2-5A is a series of oligoadenylates which is synthesized from ATP by 2-5A synthetase. The oligoadenylates are uniquely linked by 2',5'-phosphodiester

Table 2
Distribution of Enzymatically Synthesized 2-5A in Young and Old Guinea Pig Peritoneal Macrophages

Form of Enzymatically Synthesized 2-5A	Relative Distribution of 2-5A (%)			
	Young Macrophages		Old Macrophages	
	-IFN	+IFN	-IFN	+IFN
Dimer	34.4	54.9	23.8	31.1
Trimer	35.0	26.7	30.7	32.4
Tetramer and Higher Oligomers	30.6	18.4	45.5	36.4

bonds and range in size from a dimer to a pentadecamer (11,25). Investigations on the biological properties of various forms of 2-5A show that the tetramer and pentamer are more active than the trimer (26). It is of interest to determine the distribution of enzymatically synthesized 2-5A in young and old macrophages in the presence of interferon. Accordingly, 2-5A is synthesized, treated with bacterial alkaline phosphatase, and chromatographed on PEI plates to separate the different oligomeric forms of 2-5A. Results of a typical experiment are shown in Table 2. In young macrophages, there is an equal distribution between the dimeric, trimeric, tetrameric and higher oligomeric forms of 2-5A. Interferon treatment preferentially enhances the synthesis of the dimeric 2-5A while decreases the tetrameric and higher oligomeric forms of 2-5A (Table 2). In the case of the old macrophages, there is a significant increase in the percentage of the tetrameric and higher forms of 2-5A. This result may be explained by assuming that young and old macrophages have multiple forms of 2-5A synehetase that are capable of synthesizing different sizes of 2-5A.

Thermostability of 2-5A Binding Protein Activity in Young and Old Macrophages. Previously we have demonstrated that the 2-5A binding protein activity shows a marked dependence on preincubation of cell extracts at different temperatures (23). To ascertain whether macrophages from young and old guinea pigs may have the same thermostability, extracts from the two age groups are first incubated at 4°, 23°, 30°, and 37° for 30 minutes

Table 3
Effect of Preincubation at Different Temperature on 2-5A Binding Protein Activities in Young and Old Guinea Pig Macrophage Extracts

Preincubation Temperature (C)	2-5A Binding Activity (fmole/mg/h)			
	Young Macrophage		Old Macrophage	
	-IFN	+IFN	-IFN	+IFN
4	222	414	183	194
23	218	430	172	185
30	212	394	126	113
37	86	320	52	65

and then mixed with the radioactive 2-5A probe. Following an additional 60 minutes on ice, binding is quantitated by retention of the 2-5A:binding protein complex on nitrocellulose filters. As shown in Table 3, binding in young macrophages is not affected by preincubation at temperatures up to 30°C. When lystates are first incubated at 37°C, a 61% drop in binding activity is noted. As reported previously, interferon-treated macropahges from 6 month old guinea pigs are more resistant to thermal inactivation (23). Even 37°C, only a 23% decrease in binding activity is observed (Table 3). Macrophage extracts from 22-24 month old guinea pigs exhibit a more significant drop in binding activity when the lysates are preincubated at different temperatures, regardless of whether the cells have been treated with interferon.

In summary, our results show that the 2-5A synthetase and the 2-5A binding protein in aged animals have a significant reduction in their ability to respond to the challenge of interferon. This may be due to reduced receptor numbers associated with aged cells, or may be attributed to a decline in transcriptional and/or translational capacity in the process of ageing. Whatever the mechanism, these observed changes may be part of the biochemical events that are involved in the decrease in immune competence to viral infection during senescence (1,2).

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