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Emergence of New Antigens in Ageing Tissues

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Abstract. Ageing both in mice and the human is associated with the emergence of new antigenic determinant(s) which may be recognized by injecting the tissues into guinea pig and studying the sensitization developed by the animal's lymphocytes, to normal and scrapie mouse brain or spleen. New antigenic determinant(s) appear in ageing tissues sufficiently like those found in scrapie mouse brain or spleen to give cross reactions with the latter. It is possible to use a preparation of lymphocytes or RBC as a 'biopsy' material to assess the position of an individual with respect to the emergence of the antigenic determinant(s) associated with ageing. The method might be used to study the influence of disease in general upon ageing in tissues.

Key Words
Antigenic determinants
Mouse
Human
Lymphocyte sensitization

Similarities exist between certain morphological changes in the brain in scrapie, kuru and Creutzfeldt-Jakob disease an the one hand and those which occur normally in old age on the other [FIELD, 1967]. It was pointed out that in scrapie the artificially induced disease in young animals gave rise to changes which where normally to be expected only at an advanced age so that the disease appeared to have telescoped time changes in the brain and accelerated the ageing changes in the organ. More recently FIELD and SHENTON [1972] have reported that tissue from scrapie mice when injected into guinea pigs induced lymphocyte sensitization to scrapie brain or spleen in much higher degree than to corresponding normal tissues so that this procedure could be used to establish the presence of scrapie in an inoculated tissue. Because of this altered antigenicity of scrapie tissue, the antigenicity of aged tissue as

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compared with young tissue was studied and has been found that in both mice and men changes similar to those in scrapie are found with advancing age.

Materials and Methods

Mouse Experiments

Webster-Swiss mice of a long inbred strain have been used throughout. Animals of increasing age were killed, 10^{-1} suspensions of brain, spleen, liver, kidney and muscle made up in sterile polyfusor saline and cleared by centrifugation at 1,800 *g* for 10 min. 0.1 ml of such suspension was injected intracutaneously into the dorsum of the right foot of a Hartley guinea pig (of either sex and about 500–600 g body weight). Eight days later about 8 ml of blood was withdrawn by cardiac puncture and lymphocytes separated by the method of COULSON and CHALMERS [1967] as modified by HUGHES snd CASPARY [1970]. Viability was determined by the erythrosin exclusion method and final suspension prepared with 1.0 million cells in 1 ml.

Human Materials

Preliminary experiments showed that formalin fixed material was as suitable for this study as fresh and so the former has been used systematically. Material was obtained from patients who died with an acute illness (e.g. subarachnoid haemorrage, bronchopneumonia following? encephalitis lethargica, generalized peritonitis after perforated gastric ulcer) and with ages varying from 20 to 91 years. Material was washed free from formalin and made up to a 10^{-1} suspension and injected as before. In addition tests were carried out using 10^7 lymphocytes from healthy humans of different ages as a 'biopsy' of their tissues by injecting into guinea pigs as before. In two experiments erythrocytes also were used as inocula.

Lymphocyte Sensitization

Measurement of lymphocyte sensitization was made by the macrophage electrophoretic migration (MEM) test [FIELD and CASPARY, 1970, 1971; CASPARY and FIELD, 1971]. In principle the method depends upon the observation that when sensitized lymphocytes are brought into contact with antigen a macrophage slowing factor (MSF, which may be identical with MIF) is produced with the property of causing normal guinea pig macrophages to travel more slowly in an electric field. Normal macrophages are thus used as an indicator system for lymphocyte-antigen interaction.

Normal guinea pig macrophage exudate was raised by intraperitoneal injection of 20 ml sterile liquid paraffin and washing out with heparinized Hanks' solution 6–8 days later. The exudate cells were washed in Hanks' solution and finally suspended in medium 199 (without heparin) at a concentration of 10^7/ml. Since peritoneal exudate contains some 10–20% of lymphocytes the exudate was exposed to 100 rad γ-irradiation from a cobalt bomb in order to incapacitate them (at least temporarily) from taking part in a mixed reaction with human lymphocytes.

As test 'antigens' scrapie mouse brain and spleen were used. These were 10^{-1} suspension of the organs taken from a Webster-Swiss mouse inoculated intracerebrally 5 months previously with mouse-adapted scrapie (kindly supplied in the first place by Dr. R.L. CHANDLER and since passaged in this unit). 'Normal' antigen was made from brain and spleen of a mouse similarly inoculated with normal brain suspension.

Table I. Guinea pig lymphocyte sensitivity to scrapie and normal tissues immunized with mouse tissues of different ages

Age days	EF	Normal brain	Scrapie brain	Normal spleen	Scrapie spleen	Brain SND	Spleen SND
Brain injected							
27	9.2	9.4	10.4	4.0	5.2	1.2	1.0
42	7.7	7.9	9.1	4.7	6.0	1.2	1.3
42	8.1	8.3	9.6	4.9	6.1	1.3	1.2
180	12.0	9.9	12.1	4.0	6.2	2.2	2.2
203	12.5	9.9	12.4	4.4	7.0	2.6	2.5
570	9.6	9.4	13.2	4.4	9.4	3.8	5.0
840	9.7	9.7	13.8	4.3	9.1	4.1	4.8
1,050	–	9.3	13.1	4.3	8.7	3.8	4.4
Spleen injected							
27	3.7	3.8	5.0	8.9	10.1	1.2	1.2
203	5.2	4.3	6.8	9.2	11.8	2.6	2.5
570	3.9	4.1	9.1	9.1	13.6	5.0	4.5
840	4.4	4.2	9.0	8.8	12.8	4.8	4.0
1,050	–	2.8	7.6	9.6	13.2	4.8	4.6
Liver injected							
27	–	4.4	5.5	4.4	5.4	1.0	1.1
203	–	4.4	6.9	4.4	6.7	2.3	2.5
590	2.0	3.9	7.6	3.2	7.2	3.7	4.0
840	2.5	3.9	8.9	2.9	7.5	5.0	4.6
Kidney injected							
27	–	4.4	5.3	4.2	5.2	1.0	0.9
203	–	4.6	7.0	4.3	6.9	2.6	2.4
590	4.1	4.0	7.7	3.4	7.0	3.7	3.6
840	4.3	4.4	8.0	3.7	7.7	3.6	4.0

EF = encephalitogenic factor. SND is the difference in percentage slowing when scrapie brain (or spleen) is used as test antigen in the MEM test as compared with the slowing when normal brain (or spleen) is used.

In carrying out a test 0.5 million lymphocytes were mixed with 0.1 ml of antigen (10^{-1} tissue suspension) and 10^7 irradiated macrophages added. As control, macrophages were mixed with lymphocytes without the presence of antigen. As additional control to eliminate any direct slowing effect of antigen upon macrophages, another tube was put up comprising macrophages and antigen alone for each batch of the latter. Mixtures were

Table II. Guinea pig lymphocyte sensitivity to scrapie and normal mouse brain and spleen after immunization with different human tissues

Age	Sex	Normal brain	Scrapie brain	Normal spleen	Scrapie spleen	Brain SND	Spleen SND
Normal brain injected							
20	F	9.2	10.8	4.5	5.4	0.9	1.1
30	F	9.3	10.3	3.9	5.3	1.4	1.0
52	F	9.1	12.9	5.8	9.4	3.8	3.6
72	F	9.6	14.3	5.7	11.1	4.7	5.4
91	F	9.2	13.5	6.0	10.9	4.3	4.9
Spleen injected							
30	F	4.3	5.3	9.8	10.4	1.0	0.6
91	F	3.9	10.5	9.3	14.8	6.6	5.5
Heart injected							
30	F	4.3	5.1	4.3	5.2	0.8	0.9
91	F	4.2	9.0	4.1	8.4	4.8	4.3
Liver injected							
30	F	4.3	5.1	4.0	5.0	0.8	1.0
91	F	4.5	10.2	4.4	10.3	5.7	5.9
Kidney injected							
30	F	4.2	5.2	4.2	5.0	1.0	0.8
91	F	3.5	8.8	4.4	9.1	5.3	4.7

incubated at 20±2°C for 90 min and the migration speed of the macrophages then measured in a Zeiss cytopherometer. All measurements were limited to macrophages readily recognized under phase contrast illumination by their content of liquid paraffin droplets. Cells which just about filled 1 eyepiece grid square (16 μm) were selected and times which differed by more than 10% in both directions of the potential difference were recorded but not used in the calculation. Such variations are due to turbulence, departure from the electrically neutral measurement plane or minute mechanical drift. Ten cells were measured in either direction so that 20 readings were available to calculate a mean and standard deviation of the migration time over 1 square distance (16 μm).

If t_e = migration time when antigen present; t_c = time without antigen; then $t_e > t_c$ and

$$\frac{t_e - t_c}{t_c} \times 100$$

is a measure of the slowing and so of lymphocyte sensitization. Full experimental details together with a protocol *in extenso* have been given by CASPARY and FIELD [1971].

Table III. Guinea pig lymphocyte sensitivity to normal and scrapie mouse brain and spleen after immunization with human blood cells

Age	Sex	Normal brain	Scrapie brain	Normal spleen	Scrapie spleen	Brain SND	Spleen SND
White blood cells							
16	F	5.0	5.9	4.4	5.1	0.9	0.7
20	F	4.55	5.3	4.5	5.2	0.75	0.7
27	M	4.5	6.1	4.1	5.8	1.6	1.7
34	M	4.5	6.6	4.2	6.5	2.1	2.3
41	F	4.5	7.8	4.5	7.7	3.3	3.2
45	F	4.6	8.2	4.5	8.4	3.6	3.9
56	F	4.1	7.8	4.5	8.3	3.7	3.8
57	M	9.6	5.4	4.5	8.7	4.2	4.2
74	M	4.5	8.8	4.6	8.8	4.3	4.2
81	M	4.1	9.6	4.4	9.7	5.5	5.3
93	F	5.9	12.1	4.4	10.2	6.2	5.8
96	M	5.5	12.1	4.6	11.3	6.6	6.7
Red blood cells							
16	F	3.95	5.0	4.0	5.0	1.05	1.0
93	F	5.1	10.3	4.6	9.6	5.2	5.0

Results

Inoculation of Mouse Tissues

The response of the guinea pig lymphocytes to testing with normal brain or spleen was more or less constant for any particular inoculum (brain, spleen, liver, kidney) and independent of the age of the animal from which the inoculum had been prepared. The age of the immunizing tissue thus appeared to be without effect upon the lymphocyte response to normal mouse brain or spleen. However, the position was very different if scrapie mouse brain or spleen was used to test lymphocyte reactivity. Here the response clearly increased with the age of the animal from which the inocula had been prepared to immunize the guinea pig. As a result the scrapie normal difference in response (SND) rose with age (table I, fig. 1) It is clear, too, that the increase in SND is greatest in the case of the spleen and least for kidney whether testing is done with scrapie mouse brain or spleen.

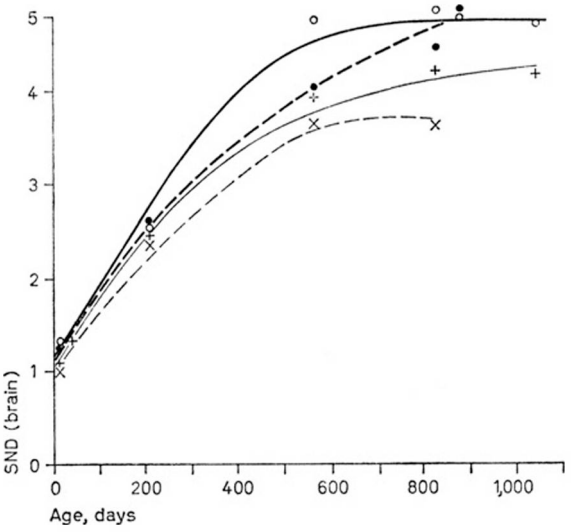

Fig. 1. Guinea pig lymphocyte sensitization to scrapie and normal mouse brain injected with Mouse tissues of different ages. - - - - - = mouse liver injected, ●; ——— = mouse spleen injected, ○; ——— = Mouse brain injected, +; ------ = mouse kidney injected, ×.

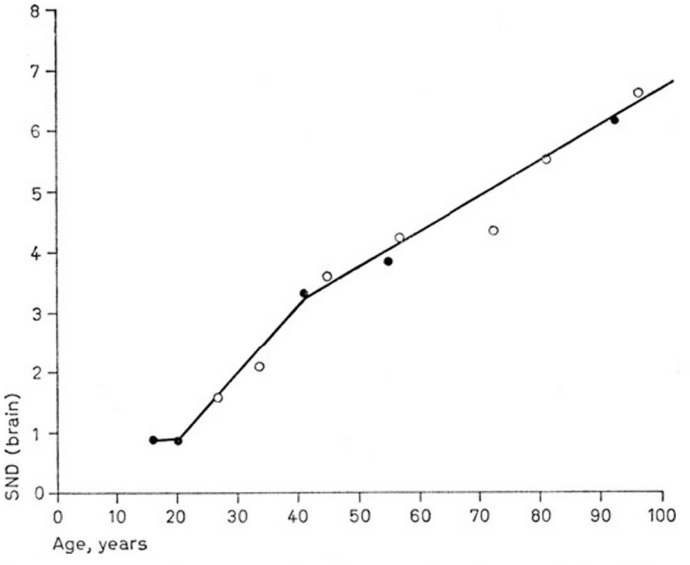

Fig. 2. Guinea pig lymphocyte sensitivity to scrapie and normal mouse brain after immunization with human peripheral lymphocytes. Female lymphocytes (●), male lymphocytes (○).

Human

Formalin-fixed tissues. The same general rise in SND was found in the responses given by lymphocytes from guinea pigs inoculated with human material of different age. Again, the increased SND was referable to higher reactivity vis-à-vis scrapie tissue (brain or spleen), rather than normal antigen responses to the latter remaining more or less independent of the age of the inoculum (table II). However, SND in the case of the human material was greatest for spleen and liver and least for brain and heart muscle.

Lymphocytes. As a 'biopsy' material lymphocytes isolated from normal human subjects were isolated and 10^7 cells inoculated into guinea pigs. It is clear that such a tissue specimen also induces an increased SND with age of the subject from whom the cells were derived. Once again the response to normal brain or spleen as test antigen does not vary with age, but that to scrapie mouse brain or spleen goes up. (table III, fig. 2)

Red blood cells. Where lymphocytes (10^7) were injected instead of lymphocytes the same trend with age was observed (table III).

Discussion

A previous study [FIELD and SMITH, 1972] has shown that lymphocytes show minor but clearly increased sensitization to autogenous antigens such as encephalitogenic factor as the subject ages, though it never exceeds 5%. When, however, these same lymphocytes are inoculated into a guinea pig, the animal recognizes that they have become antigenically altered with the passage of time and the same is apparently true of other tissues of the body. The ageing of the tissue is apparently associated with the emergence of antigenic determinant(s) similar to those found in scrapie tissues [FIELD and SHENTON, 1972, 1973a, b] and such new determinant(s) are found even on erythrocytes. The change appears to be ubiquitous in the tissues. Whilst there is at this stage no certain knowledge of the location of the new determinant(s), analogy with cancer cells might suggest it is associated with the plasmalemmal membrane rather than with endoplasmic reticulum or nucleus [DICKINSON *et al.*, 1972]. It remains to isolate the new material and determine its true relation to scrapie antigen(s) – a problem which might well respond to the combination of affinity chromatography and cytopherometric lymphocyte sensitization adopted for studying the antigenic relationship between measles virus and encephalitogenic factor [FIELD and MCDERMOTT, 1973].

Surface changes in cells may well be of importance in maintaining the organic cohesion of a tissue, a factor which could be impaired in ageing.

It is clear that the emergence of the new antigenic determinants, whatever their nature, appears to occur rather more quickly in liver tissue than in kidney. A similar ageing phenomenon has recently been observed in neonatally thymectomized mice and again the antigenic changes in liver are greatest [FIELD and SHENTON, 1973b, c]. Minor differences emerge also in the responses to various tissues from ageing humans (table II), though they are not so clear as in mouse tissues.

Throughout it will be seen that the response to normal tissue remains relatively unchanged with age of the tissue but it is the lymphocyte reactivity to scrapie tissue which increases producing an exaggerated SND.

If further work establishes the present technique as a reliable immunological method of assessing ageing, then it may be useful for comparison of calendar with physiological ageing and evaluating conditions which accelerate or delay the ageing process. Of special interest would be a study of subjects from those areas of the world, e. g. Georgian, Republic Ecuador, where great age is reported to be commonly attained.

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