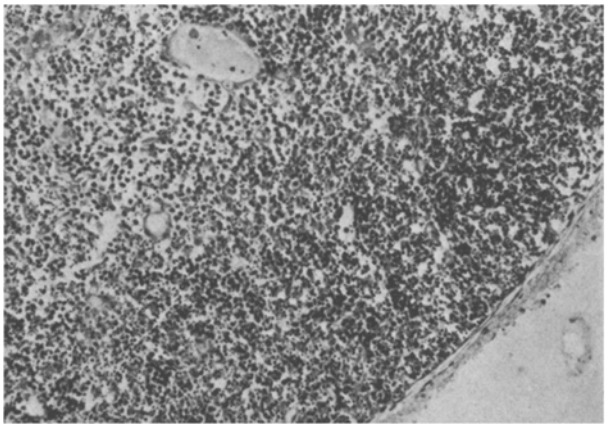

Fig. 3. Karyorrhexis in the thymic cortex of a neonatal mouse 24 h after the i.p. administration of salivary gland homogenate. ×40.

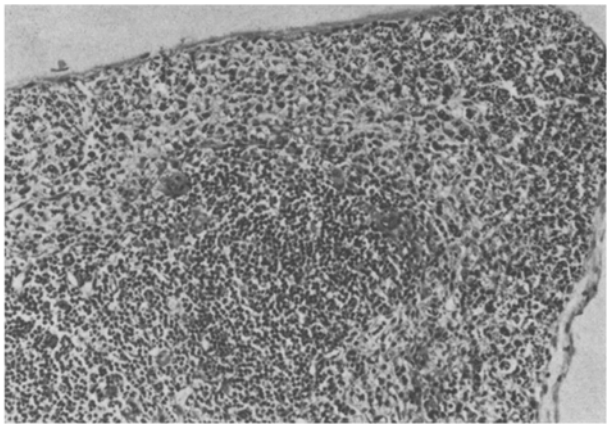

Fig. 4. Thymic cortex devoid of intact lymphocytes 48 h after the i.p. administration of salivary gland homogenate. ×40.

lymphocytes. It appears that they disintegrate and migrate into the medulla[12]. The 2 cell compartments concerned correspond to the cortisol-sensitive and cortisol-resistant thymocyte subpopulation[13]. Under the influence of salivary gland homogenate, the cortisol-resistant thymocytes seem to migrate into the medulla at a faster rate. This is in agreement with the finding that cortisol-sensitivity in mice is age-dependent and not only is cytotoxicity different but also the rate of migration. It is not clear whether the various factors demonstrated (e.g. the epidermal growth factor) are present exclusively in the salivary gland, or if they only occur there to a greater extent[15].

On the one hand, thymic tissue proliferates after removal of the submandibular glands of adult mice; on the other hand, salivary gland homogenate has a suppressive action on the thymus in both adrenalectomized and gonadectomized mice, showing that the atrophic effects were not mediated by these organs[8]. Thus it cannot be ruled out that there is a substance in the salivary gland which has a cortisol-like effect and which regulates the cell kinetics of the thymus.

12 I. L. Weissmann, J. exp. Med. *137*, 504 (1973).
13 M. Schlesinger and E. Israël, Cell Immun. *18*, 144 (1975).
14 M. H. Claesson and V. Tjell, Acta Path. microbiol. scand. Sect. C *84*, 185 (1976).
15 M. L. Roberts, J. A. Freston and P. C. Reade, Immunology *30*, 811 (1976).

Effect of ageing on surface IgG of human peripheral lymphocytes

C. Zanussi, C. Rugarli, B. Perussia* and R. Scorza Smeraldi

Istituto di Clinica Medica IV, Padiglione Litta-Policlinico dell'Università, via Francesco Sforza 35, I-20122 Milano (Italy), 26 November 1976

Summary. An increase in the percentage of IgG bearing peripheral blood lymphocytes is observed in aged subjects as compared with young ones. Such a finding is probably due to the presence, in 'aged' sera, of a higher concentration of immune complexes, bound to lymphocytes through their Fc or Complement receptors.

The receptor function of IgM and IgD on B-cell membrane is well assessed[1-4], whilst IgGs are commonly considered as artifacts[5-7]. This evidence casts doubt on the significance of the age-associated increase of the percentage of IgG bearing lymphocytes observed in healthy humans[8]. A brief in vitro culturing of lymphocytes (1h) is useful to clear the cells from passively attached molecules and, for instance, to free them from the bulk of membrane IgG[7] without affecting the expression of the true membrane receptors. Thus we studied the effect of a 'shedding and regeneration' treatment on peripheral lymphocytes from young and aged donors, and we controlled the effect of the incubation with blood serum on the recorded values.

Materials and methods. Peripheral lymphocytes were harvested from heparinized venous blood according to the technique quoted by Aiuti et al.[9] from 10 healthy young volunteers (less than 30 years old) and 10 aged patients suffering from minor cardiovascular complaints (more

* Present address: Ludwig Institute for Cancer Research. CH-1066 Épalinges s/Lausanne, Switzerland.

1 Membrane receptors of lymphocytes. Ed. M. Seligmann, J. L. Preud'homme and F. M. Kourilsky. North-Holland Publishing Co., Amsterdam 1975.
2 E. S. Vitetta, U. Melcher, M. McWilliams, M. E. Lamm, J. M. Phillips Quagliata and J. W. Uhr, J. exp. Med. *141*, 206 (1975).
3 D. Rowe, K. Hug, L. Forni and B. Pernis, J. exp. Med. *138*, 965 (1973).
4 E. S. Vitetta, J. Forman and J. R. Kettman, J. exp. Med. *143*, 1055 (1976).
5 E. S. Vitetta and J. W. Uhr, Science *189*, 964 (1975).
6 S. M. Fu, R. J. Winchester and H. G. Kunkel, J. exp. Med. *139*, 451 (1974).
7 R. J. Winchester, J. B. Winfield, F. Siegal, P. Wernet, Z. Bentwich and H. G. Kunkel, J. clin. Invest. *54*, 1082 (1974).
8 H. A. Del Pozo Perez, J. Prieto Valtuena, M. I. Gonzales Guilabert and R. Velasco Alonso, Biomedicine *19*, 340 (1973).
9 F. Aiuti, J.-C. Cerottini, R. R. A. Coombs et al., Clin. Immun. Immunopath. *3*, 584 (1975).

Percentage (mean ± standard error) of slg bearing peripheral blood lymphocytes from young and aged subjects

Membrane direct immunofluorescence percent (mean ± SE) of lymphocytes positive for:	Subjects Age < 30 years (10 cases) A	B	C	Age > 70 years (10 cases) A	B	C
IgG	8.54 ± 0.70	3.43 ± 0.30 $p < 0.01$	9.75 ± 1.35 $p < 0.01$	10.56 ± 0.94	6.57 ± 0.52 $p < 0.01$	14.98 ± 1.93 $p < 0.01$
IgM	7.69 ± 1.57	8.32 ± 1.79 n.s.	13.33 ± 2.15 $p < 0.01$	6.43 ± 0.88	12.34 ± 0.85 $p < 0.005$	13.95 ± 1.56 n.s.
IgD	5.52 ± 0.79	3.16 ± 0.83 $0.02 > p > 0.01$	3.64 ± 0.53 n.s.	3.58 ± 0.52	4.31 ± 1.44 n.s.	2.43 ± 0.95 n.s.
IgA	3.04 ± 0.49	1.54 ± 0.41 $0.05 > p > 0.02$	3.02 ± 0.42 $0.02 > p > 0.01$	1.89 ± 0.41	2.71 ± 0.54 n.s.	3.02 ± 0.42 n.s.

A, basal values; B, values after 'shedding' (1 h culture at 37 °C); C, values after post-shedding incubation with autologous sera. p was calculated on the basis of the Student's t-test. In B the mean of the differences (± SE) between basal and post-shedding values were calculated; in C we calculated the mean of the differences (± SE) between shedding and post-shedding incubation with autologous sera values – n.s., not significant.

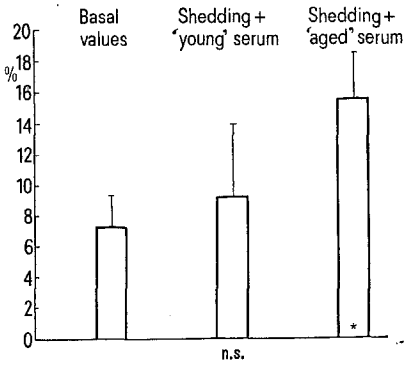

Fig. 1. Percentages (mean and SD) of IgG bearing peripheral lymphocytes: effect of post-shedding incubation with homologous serum from young and aged donors. *: mean of the differences from basal values significant at $P < 0.001$ level. n.s., the same not significant.

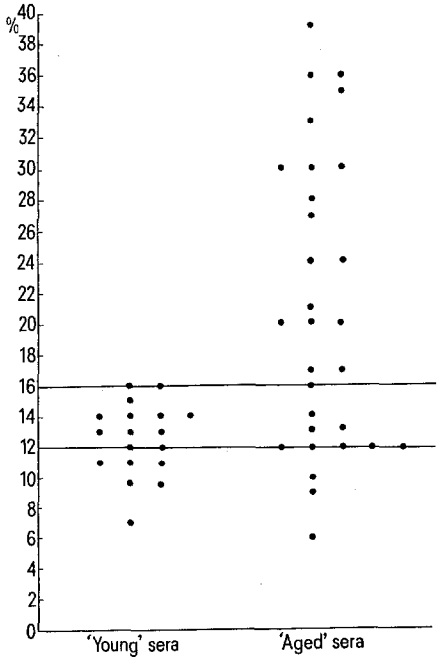

Fig. 2. C1q binding test.

than 70 years old). Yields between 95 and 98% viable lymphocytes (by the Trypan blue exclusion test) were common. Surface Ig were detected by direct immunofluorescence staining according to Loor et al.[10]. FITC-conjugated goat anti-human Ig classes antisera (Cappel), cleared from aggregates by centrifugation at 25,000 × g for 4 h, were used. Specificity controls were done by immunoelectrophoresis and direct staining of smears of myeloma (IgG/k) and Waldenström bone marrow aspirations. Prior to the staining, the lymphocytes were allowed to 'shed' by a 2-h culturing (10^6 cells/ml) in Tc 199-Hepes (Eurobio) plus 1% L-glutamine (Eurobio), 20% fetal calf serum (FCS Eurobio) and antibiotics (penicillin 100 U/ml and streptomycin 100 µg/ml) followed by 2 washings in PBS-BSA 2%-NaN_3 0.1% pH 7.2. In some instances, the cultured lymphocytes were incubated (1 h at 4 °C) with autologous and homologous blood sera, washed twice in PBS, and then tested for surface Ig.

The blood sera were previously tested for cytotoxic antibodies on autologous and homologous lymphocytes, both at 37 °C and at 4 °C, according to Mittal et al.[11]. No consistent positivity of this test was found either in young or in aged donors. The blood sera from other 18 young volunteers and 30 aged donors were employed for detecting antigen-antibody complexes in the ^{125}I-C1q binding test according to Zubler et al.[12].

Results and discussion. The table reports the percentage of Ig bearing peripheral lymphocytes both from young and aged donors. No major difference is noticeable in basal values between age groups, and a significant fraction of lymphocytes is positive for IgG staining. When the cells were induced to 'shed', a marked decrease in the percentage of IgG bearing lymphocytes was noted in both the groups. Beyond this change, only an increase in the percentage of IgM bearing lymphocytes from aged donors was significant at the $P < 0.005$ level. The incubation with autologous serum restored the percentages of IgG bearing cells to the pre-shedding values in both of the groups. The increase in IgM positive cells became significant also in the young group. Both after shedding and after shedding plus incubation with autologous serum, the

10 F. Loor, L. Forni and B. Pernis, Eur. J. Immun. *2*, 203 (1972).
11 K. K. Mittal, M. R. Mickey, D. P. Singal and P. I. Terasaki, Transplantation *6*, 913 (1968).
12 H. H. Zubler, G. Lange, P. H. Lambert and P. A. Mischer, J. Immun. *116*, 232 (1976).

levels of IgG bearing lymphocytes in aged donors were higher than in young controls at the corresponding experimental stages. Figure 1 reports the effect of blood serum, both from young and aged donors, on the percentage of IgG bearing cells in a group of homologous young normal lymphocytes previously submitted to the 'shedding' procedure. Only after incubation with 'aged' serum was such a value enhanced at a significant level. Figure 2 depicts the values of C1qbinding activity of blood sera from young and aged donors: 19 out of 30 'aged' sera exhibited a significant binding activity, whilst no positivity was recorded among the 'young' sera.

2 main results were observed concerning Ig bearing peripheral lymphocytes after in vitro culturing. An increase in the percentage of IgM bearing cells took place both in young and in aged donors. A similar increase is evident when lymphocytes are stimulated with PHA[13,14]; thus we assume that a better expression of the true receptor molecules on B-lymphocytes may be due to their stimulation in vitro. It is possible that, even if to a lesser degree, such a process is operating also during the short in vitro culturing performed (in presence of FCS) in our 'shedding' process. The second effect observed was a decrease in the percentages of IgG bearing lymphocytes both in young and in aged donors. Such a result suggests that serum born IgG are passively attached at least on a fraction of peripheral lymphocytes. The possibility that both shedding and regeneration processes of IgG receptors may exhibit age-related changes seems to be unlikely if we take into account the effect of the incubation with blood sera. Thus the age-associated differences in the percentage of IgG positive lymphocytes may be attributed to a higher concentration of such 'absorbable' IgG in blood serum from aged subjects. The effect of serum from aged donors on lymphocytes from young subjects is consistent with such a possibility. 2 possible explanations can be advanced; firstly, anti-lymphocyte autoantibodies may be present in blood sera[7,15–17]. However the blood sera employed were devoid of such antibodies, at least as far as they are detectable by the lymphocytotoxicity test. An alternative explanation assumes that antigen-antibody complexes may be bound on lymphocytes through their Fc or Complement receptors[18–20]. Should this be the case, the concentration of such complexes would be higher in the sera from aged donors. Though not conclusive, the results obtained in the ^{125}I-C1q binding test are consistent with our second hypothesis.

13 T. L. Whiteside and B. S. Rabin, J. clin. Invest. *57*, 762 (1976).
14 B. Pernis, J. F. A. P. Miller, L. Forni and J. Sprent, Cell. Immun. *10*, 476 (1974).
15 R. P. Messner, M. S. Kennedy and J. G. Jelinek, Arthritis Rheum. *18*, 201 (1975).
16 G. Biberfeld, P. Biberfeld and H. Wigzell, Scand. J. Immun. *5*, 87 (1976).
17 W. Glinski, M. E. Gershwin and A. D. Steinberg, J. clin. Invest. *57*, 604 (1976).
18 T. O. Yoshida and B. Andersson, Scand. J. Immun. *1*, 402 (1972).
19 C. R. Parish, Transpl. Rev. *25*, 98 (1975).
20 H. B. Dickler, J. exp. Med. *140*, 508 (1974).

A haemagglutinin in the tissue fluid of the Pacific oyster, Crassostrea gigas, with specificity for sialic acid residues in glycoproteins[1]

S. W. Hardy, P. T. Grant and Thelma C. Fletcher

Institute of Marine Biochemistry, Natural Environment Research Council, St. Fittick's Road, Aberdeen AB1 3RA (Scotland), 16 December 1976

Summary. An agglutinin for human red cells has a specificity for sialic acid and a high affinity for bovine salivary glycoprotein. Digestion of the glycoprotein with Pronase or neuraminidase indicated that binding of sialic acid to receptors in the agglutinin is the first step in the mechanism of formation of a stable complex between ligand and receptor.

Carbohydrate-binding proteins, the lectins, have been obtained from both plant and animal tissues[2]. The specificity has usually been determined by competitive-binding studies involving the ability of monosaccharides or their derivatives to inhibit the agglutination of red blood cells by the lectin. At the same time, it has been demonstrated that glycoproteins or glycopeptides have in many cases a significantly greater affinity for the lectin than the corresponding monosaccharide[3,4]. The reasons for this important difference remain to be defined. The present report is concerned with the occurrence and properties of the agglutinins present in the haemolymph and tissue fluids of the Pacific oyster with particular emphasis on a lectin that exhibits this difference with regard to free sialic acid and that bound as the terminal carbohydrate residues in some glycoproteins.

Tissue fluid, consisting mainly of haemolymph, was obtained by collecting the clear supernatant remaining after centrifugation (30,000 × g for 10 min) of the freshly dissected soft tissues of Crassostrea gigas. This fluid agglutinated all red blood cells of the species tested as well as other cell types including bacteria. In a standard plate assay (equal volumes of diluted tissue fluid and of a 2% v/v suspension of red blood cells in saline) typical titres obtained by serial dilution of the tissue fluid were 1/1024 and 1/256 using equine and human red blood cells respectively. These agglutinins are subsequently referred to as Gigalin H (human) and Gigalin E (equine). They are separate entities as judged by cross-absorption experiments and by the preferential absorption of Gigalin H from tissue fluid by an affinity column prepared by the reaction of CNBr – activated Sepharose 4B (Pharmacia) with a preparation of bovine salivary gland glycoprotein (BSG). The bound agglutinin could subsequently be displaced by the use of a gradient of increasing ionic strength in the eluant buffer. In addition, after treatment with neuraminidase, human red cells were not aggluti-

1 We wish to thank Dr P. Walne, Conwy Laboratory, MAFF, for generous supplies of Crassostrea gigas. One of us (SWH) wishes to thank the Natural Environment Research Council for financial support.
2 H. Lis and N. Sharon, Ann. Rev. Biochem. *42*, 541 (1973).
3 S. Toyshima, M. Fukuda and T. Osawa, Biochemistry *11*, 4000 (1972).
4 A. C. Roche, R. Schauer and M. Monsigny, FEBS Letters *57*, 245 (1975).