Conclusions. We believe that some change in the endocrine system (gonadotropin levels) and probably the phenomenon of H-Y antigen expression in the female fish influence the initiation of sex inversion, from female to male in *Coris julis.*

Reinboth[21] discusses a controlling mechanism maintaining the fish in the female sex until the beginning of the sex-reversal. On the receipt of 'impulses' (environmental or genetical factors) the role of the 'control' is altered and development of the new sex occurs. The location of this 'control' is perhaps in the CNS.

Genetic factors determine and control the development of the gonads as well as the age of sex-maturation. Possibly they also determine sex reversal in *Coris julis.* This control mechanism brings about changes in the pituitary and in the gonads as well as in H-Y antigen expression. The CNS, which also controls social interrelations between individuals may, on the one hand, be directed by these genetically determined control mechanisms, on the other hand it might influence them. Environmental factors, primarily social relationships, may play a major role in the initiation of the sex-reversal process in *Coris julis.*

1 This study was supported in part by grant 3.751.80 from the Schweizerischer Nationalfonds.
2 Acknowledgment. The authors are indebted to Mr Naseem Malik for helping with the preparation of the manuscript.
3 Duchac, B., Rapp. Com. int. Mer Medit. *27* (1981) 187.
4 Duchac, B., Thesis, University of Basel, Basel 1981.
5 Duchac, B., Huber, F., Müller, Hj., and Senn, D.G., Experientia *38* (1982) 809.
6 Reinboth, R., Zool. Anz., suppl. *24* (1961) 259.
7 Roede, M.J., Vie Milieu A *17* (1966) 1317.
8 Chan, S.T.H., Trans. R. Soc. Lond. B *259* (1970) 59.
9 Chan, S.T.H., in: Handbook of sexology, p.91. Eds J. Money and R. Musaph. Elsevier/North-Holland Biomedical Press, Amsterdam 1977.
10 Fishelson, L., in: Intersexuality in the animal kingdom, p.284. Ed. R. Reinboth. Springer, Heidelberg 1974.
11 Ohno, S., in: The evolution of reproduction, p.1. Eds C.R. Austin and B. Short. Cambridge University Press, Cambridge 1976.
12 Robertson, D.R., Science *177* (1972) 1007.
13 Fellous, M., Günther, E., Kemler, R., Wiels, J., Berger, R., Guenet, J.L., Jakob, H., and Jakob, F., J. exp. Med. *148* (1978) 59.
14 Wachtel, S.S., Biol. Reprod. *22* (1980) 1.
15 Wolf, U., Hum. Genet. *47* (1979) 269.
16 Pechan, P., Wachtel, S.S., and Reinboth, R., Differentiation *14* (1979) 189.
17 Müller, U., and Wolf, U., Differentiation *14* (1979) 185.
18 Chan, S.T.H., and Hui, W.B., in: Intersexuality in the animal kingdom, p.201. Ed. R. Reinboth. Springer, Heidelberg 1974.
19 Reinboth, R., Gen. comp. Endocr. *2* (1962) 629.
20 Reinboth, R., Mem. Soc. Endocr. *18* (1970) 515.
21 Reinboth, R., Biol. Reprod. *22* (1980) 49.

0014-4754/83/070767-03$1.50 + 0.20/0

Effect of specific and nonspecific immune complexes on injury of intact hepatocytes in vitro[1]

H. Kawanishi

Laboratory for Gut/Liver Immunology Research, Veterans Administration Medical Center and Georgetown University Medical School, Washington, D. C. 20422 (USA), November 8, 1982

Summary. Immune complex (IC)-mediated cytotoxicity of hepatocytes was investigated in vitro, using antibodies against specific and nonspecific target cell surface membranes. The results indicated that neither specific, nor nonspecific IC evoked significant lysis of intact hepatocytes. Thus, the IC are unlikely to play an important role in the immune-associated injury of normal hepatocytes in acute and chronic liver diseases.

Sera from patients with hepatitis B virus (HBV)-induced acute and chronic liver diseases contain HBV-specific immune complexes (IC)[2], as well as antibodies to liver membrane antigens[3]. Circulating immune complexes have also been demonstrated in patients with primary biliary cirrhosis[4] and alcoholic hepatitis[5]. Multiple nonspecific bacterial and dietary antigens, their antibodies, and IC originating from the gastrointestinal tract may appear, when the function of hepatic reticuloendothelial system is diminished, such as seen in chronic liver disease[6]. Furthermore, immunoglobulins (Ig), as well as IC, are also present in the livers[7,8]. In vivo fixed IgG is demonstrable on hepatocytes from animals with experimentally produced chronic active hepatitis (CAH) and from patients with CAH[9]. Interestingly, complement (C′) is found in vitro to be fixed on liver cells from patients with acute B-viral hepatitis[10]. Despite extensive studies of the induction of hepatocyte damage in vitro by cell-mediated immune mechanisms[9], such as cytotoxic lymphocyte-mediated hepatocytolysis in CAH[11], it remains unclear whether in vivo antibodies, C′ and IC injure liver parenchymal cells in acute and chronic liver diseases. Thus, in the present study, we examine the possible role of these humoral products in the pathogenesis of hepatocyte damage in vitro.

Materials and methods. Rat hepatocytes were isolated by collagenase perfusion[12]. The medium used in these experiments was RPMI 1640 and 10% heat-inactivated fetal calf serum, supplemented with streptomycin, penicillin, fungizone, 10 mM HEPES and 2 mM L-glutamine (Grand Island Biologicals, Grand Island, N.Y., USA). Six antisera (Ab) were produced through rabbits; anti-human liver-specific protein (LSP)[13], anti-rat hepatocyte (RH)[14], anti-Chang cell[14], anti-T cell (JM-T)[14], and anti-Null cell (NALM −16)[14] (both of the last 2 cell lines are those of human leukemia cells), and anti-sheep red cell (SRBC) hemolysin (Cappel Laboratories, Cochranville, PA, USA). Human liver tissues for the preparation of human LSP were obtained within 4 h after death of 2 cases, who did not have any liver injury macroscopically and histologically. The antisera were all heat-inactivated (56 °C for 45 min). A titer of anti-human LSP was determined by passive hemagglutination (1:6400)[15]. Titers of the other antisera were measured, using antibody-dependent cell-mediated cytotoxicity (optimal dilutions, 1.2×10^3 to 4×10^3). The specificity of each Ab was also preliminarily tested by indirect immunofluorescence; each had the cell membrane antibody against each corresponding cell type, including the anti-LSP which expressed its antigen-binding capacity on

the surface of the enzymatically isolated hepatocytes. In addition, human chromatographically purified IgG (Cappel Laboratories) was heat-agglutinated to obtain a nonspecific polymeric antibody (HAIgG). Activity of 2 complement (C′) was pretested by a SRBC hemolysis assay (1:140)[16]. In some experiments, SRBC were precoated with LSP[17]. ^{51}Cr release microcytotoxicity assay was used to determine IC-mediated target cell lysis[18]. The percent cytotoxicity was calculated as follows:

$$\frac{\text{Experimental release (cpm)} - \text{Spontaneous release (cpm)}}{\text{Maximal release (cpm)} - \text{Spontaneous release (cpm)}} \times 100$$

Various incubation periods were tested, ranging between 30 min and 18 h. The final dilutions of antisera and C′ were 8–20×10^3, and 1–20×10^2, respectively. All concentrations of antiserum, C′, heat-inactivated C′ (56 °C for 45 min) or HAIgG alone, antiserum plus heat-inactivated C′, HAIgG plus C′ or heat-inactivated C′ used were not cytotoxic to each target ($p > 0.05$). The results were expressed as percent mean values of 3 experiments in triplicate ± SEM. Statistical significance was determined by the Wilcoxon-test, using the sum of ranks (p).

Results and discussion. As shown in the table, IC consisting of antibody against human liver-specific membrane lipoprotein (LSP) (species-nonspecific)[13] or rat hepatocytes and C′ (fresh guinea-pig serum or zymosan activated human AB serum) did induce killing, though minimal, of isolated hepatocytes ($p < 0.001$). Other antiserum, antigen and C′ complexes did not provoke target hepatocyte damage ($p > 0.05$). The similar results, but with more significant cytotoxicity (31 ± 4%, 12 h) were obtained, when Chang cells as the target were reacted only with anti-Chang cell antibody, but not with other antibodies (data not shown). IC-mediated cytotoxicity against either LSP-coated or uncoated SRBC (table), and also against human leukemia cell lines with the corresponding Ab was rapid and complete.

The present demonstration of only slight hepatocytotoxicity by specific IC and the absence of any significant cytolytic changes of hepatocytes mediated by nonspecific IC, specific or nonspecific antibodies alone, as well as C′ alone raised the question of the etiological importance of IC as a cause of primary hepatocyte damage.

It has been postulated that reduced phagocytosis by Kupffer cells may cause the increased deposition of IC on and around hepatocytes subsequently inducing hepatocellular injury in vivo[7,8]. On the other hand, normal mammalian hepatocytes bear receptors for the Fc fragment of IgG, C_3[19] and oligomeric IgA[8]. These cells can also transport polymeric IgA and IgM and possible some IgG[20]. Thus, hepatocytes appear capable normally of clearing not only C′, Ig, but also IC. It is possible, however, that preexisting hepatocyte injury such as that induced by hepatocytolytic lymphocytes in cases with CAH[11], may be accentuated by deposits of IC, as demonstrated in animals[21].

In contrast, IC composed of HBV or hepatocyte surface membrane antigenes and antibodies may be important in the hepatocyte-effector cell interaction by interfering with cell-mediated hepatocyte lysis. This hypothesis is supported by the observation that humoral blocking factors are present in sera of experimental animals and humans with malignant disease and may inhibit or abolish tumor cell destruction by effector lymphocytes; such blocking substances appear to be composed primarily of tumor antigen-antibody complexes[22]. Thus, it seems quite possible that IC may play a protective function in vivo, regulating the immune response of hosts with liver damage caused by immune lysis of hepatocytes.

It has here been demonstrated in vitro that intact hepatocytes are largerly resistant to damage by specific and nonspecific IC and its components. Also, specific antibodies or C′ alone are not responsible for primary hepatocytolysis. Thus, both specific and nonspecific IC in vivo probably do not play major roles in the initiation of target hepatocyte destruction in acute and chronic immune-associated liver diseases.

Immune complex-mediated cytotoxicity in vitro of target hepatocytes and other target cells

Experimental system using different target cells	Percent maximal cytotoxicity (^{51}Cr release)	Optimal incubation Time (h)	p-Value[a]
Rat hepatocytes			
Anti-LSP[c] + GC′[b,c]	11 ± 2	6	< 0.001
Anti-LSP[c] + ZC′[b,c]	10 ± 2	6	< 0.001
Anti-RH[c] + GC′[c]	12 ± 2	6	< 0.001
Anti-RH[c] + ZC′[c]	13 ± 2	6	< 0.001
Anti-Chang[c] + GC′[c]	1 ± 1	12	> 0.05
Anti-Chang[c] + ZC′[c]	1 ± 1	12	> 0.05
Anti-T Cell (JM-T)[c] + GC′[c]	2 ± 1	12	> 0.05
Anti-T Cell (JM-T)[c] + ZC′[c]	2 ± 1	12	> 0.05
Anti-Null Cell (NALM-16)[c] + GC′[c]	1 ± 1	12	> 0.05
Anti-Null Cell (NALM-16)[c] + ZC′[c]	0 ± 1	12	> 0.05
SRBC			
Anti-SRBC (hemolysin)[f] + GC′[d]	87 ± 4	1	< 0.001
Anti-SRBC (hemolysin)[f] + ZC′[d]	85 ± 3	1	< 0.001
LSP-precoated SRBC			
Anti-LSP[e] + GC′[d]	68 ± 2	2	< 0.001
Anti-LSP[e] + ZC′[d]	60 ± 4	2	< 0.001
Anti-RH[e] + GC′[d]	66 ± 3	2	< 0.001
Anti-RH[e] + ZC′[d]	64 ± 3	2	< 0.001

[a] p-Values against spontaneous release of ^{51}Cr at each incubation time. [b] GC′, guinea-pig serum complement; ZC′, zymosan activated human AB serum. Final optimal concentration, [c] 1:20 dilutions; [d] 1:30; [e] 1:100; [f] 1:1200.

1 Acknowledgment. This work was supported by a Veterans Administration Research Fund at Ann Arbor Veterans Administration Medical Center, Michigan, during 1977–1980.

2 Maclalinski, K., and Braziel, L., Clin. exp. Immun. *36* (1979) 371.
3 Kakumu, S., Arakawa, H., Goji, H., Kashio, T., and Yata, K., Gastroenterology *76* (1979) 665.
4 Thomas, H.C., Potter, B.J., and Shelock, S., Lancet *2* (1977) 1261.
5 Penner, E., Albini, B., and Milgrom, F., Clin. exp. Immun. *34* (1978) 28.
6 Triger, D.E., Gastroenterology *71* (1976) 162.
7 Alberti, A., Realdi, G., Tremolando, F., and Spina, G.P., Clin. exp. Immun. *25* (1976) 396.
8 Gerber, M.A., Sarno, E., and Vernace, S.J., New Engl. J. Med. *249* (1976) 922.
9 Meyer zum Büschenfelde, K.H., Hütteroth, T.H., Arnold, W., and Hopf, V., in Progress in liver diseases, p.407. Eds H. Popper and F. Schaffner. Grune and Stratton Co., New York 1979.
10 Rizzetto, M., Diana, S., Bonino, F., and Verme, G., Lancet *2* (1976) 436.
11 Wands, J.R., and Isselbacher, K.J., Proc. natl Acad. Sci. USA *72* (1975) 1301.

12 Bonney, R.J., In Vitro *10* (1974) 130.
13 Meyer zum Büschenfelde, K.H., and Miescher, P.A., Clin. exp. Immun. *10* (1971) 89.
14 MacLennan, I.C.M., Campbell, A.C., and Gale, D.G.L., in: In vitro methods in cell-mediated and tumor immunity, p.511. Eds B.R. Bloom and J.R. David. Academic Press, New York 1976.
15 Gravey, J.S., Cremer, N.E., and Sussdorf, D.H., in: Methods in immunology, 3rd edn, p.347. Eds J.S. Garvey, N.E. Cremer and D.H. Sussdorf. W.A. Benjamin, Inc., Massachusetts 1977.
16 Garvey, J.S., Cremer, N.E., and Sussdorf, D.H., in: Methods in immunology, 3rd edn, p.379. Eds J.S. Garvey, N.E. Cremer and D.H. Sussdorf. W.A. Benjamin, Inc., Massachusetts 1977.
17 Poston, R.N., J. Immun. Meth. *5* (1974) 91.
18 Brunner, K.T., Engers, H.D., and Cerottini, J.C., in: In vitro methods in cell-mediated and tumor immunity, p.423. Eds B.R. Bloom and J.R. David. Academic Press, New York 1976.
19 Hopf, V., Meyer zum Büschenfelde, K.H., and Dierich, M.P., J. Immun. *117* (1976) 639.
20 Fischer, M.M., Nagy, B., Bazin, H., and Underdown, B.J., Proc. natl Acad. Sci. USA *76* (1979) 2008.
21 Paronetto, F., and Popper, H., Am. J. Path. *49* (1966) 1087.
22 Theofilopoulos, A.N., and Dixon, F.J., in: Immunodiagnosis of cancer, part 2, p.896. Eds R.B. Herberman and K.R. McIntire. Marcel Dekker, New York 1979.

0014-4754/83/070769-03$1.50 + 0.20/0

Serum inhibitory activity on granulocyte-macrophage colony formation

M. Szmitkowski

Department of Clinical Diagnostics, Institute of Biochemistry and Medical Analytics, Medical School, PL-15-276 Białystok (Poland), May 7, 1982

Summary. Normal and chloroform-extracted human sera, fractionated by Sephadex column chromatography, have been tested for inhibitory activity on granulocyte-macrophage (GM) colony formation. It was found that this activity is connected with lipoproteins (inhibitors of granulocyte-macrophage colony stimulating factor) and low molecular weight substances (7000; 13,000) which can act as specific polypeptide chalones.

The proliferation and differentiation of granulocyte and macrophage colony forming cells in agar culture (CFC-c) is under the control of the glycoproteid substance called colony stimulating factor (CSF). Many data clearly confirm that CSF function is intimately linked with the regulation of granulopoiesis[1].

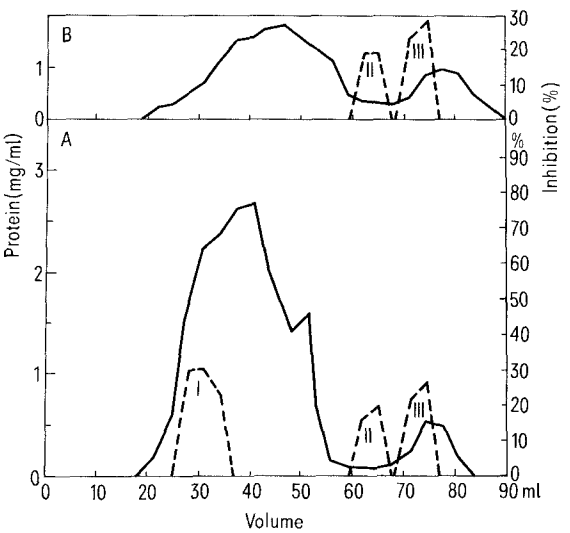
On the other hand, the growth and differentiation of CFC-c can be inhibited by several substances, such as chalones of granulopoiesis which are found in mature granulocytes, and have been precisely characterized by Paukovits et al.[2] as polypeptides with molecular weights ranging from 5000 to 10,000. An inhibitor of CSF biosynthesis has been found by Broxmeyer et al.[3,4], also in mature granulocytes. This inhibitor was characterized as lactoferrin (mol.wt 80,000–100,000, isoelectric points 6.0–6.5), an iron-binding glycoprotein found in the secondary granules, which can suppress the in vitro and in vivo production of granulocyte-macrophage colony stimulating factor by monocytes. The serum unspecific inhibitor of CSF, which has been characterized as a lipoprotein, inhibits the action of CSF directly and nonspecifically[5,6]. My attention has been directed to the study of the inhibition of GM colony formation by normal and chloroform-extracted human sera fractionated by Sephadex column chromatography.

Materials and methods. Chloroform extraction was used in order to remove the lipoproteins from sera[6]. Human sera were pooled from healthy subjects.

Chromatography procedures were carried out on a column (1.6×40 cm) with Sephadex G-200, eluted by 0.9% NaCl; about 100 mg of protein (1.5 ml of serum) was applied to the column. Protein concentration in the fraction samples was determined spectrophotometrically. Molecular weights were determined by chromatography on Sephadex G-200 and G-50.

Colony (> 50 cells/clone) formation was determined by plating 10^5 mouse bone nucleated marrow cells in 1 ml of 0.3% agar medium containing 0.05 ml of fraction sample and 0.05 ml of stimulant (1:2 diluted serum from a mouse treated with *Escherichia coli* endotoxin as an agent enhancing CSF level). Cultures were incubated at 37 °C in a humidified atmosphere containing 7.5% CO_2 in air for 1 week. Colonies and clusters were scored from 3 to 5 plates for each assay point.

Results and discussion. In normal human serum, after the chromatography procedure, the inhibitory activity of granulocyte-macrophage colony formation is distributed between 3 peaks, localized in elution volumes of 30 ml, 64 ml and 73 ml respectively (fig. A). In the chloroform-extracted human serum after chromatography, only 2 peaks were found (fig. B). They were localized in the same elution volumes as peaks II and III in normal serum. I did not find any traces of the activity of peak I in this serum. Molecular weights determined for active substances in each peak of these sera revealed the following data; peak I-200,000, II-13,000, III-7000.



Inhibition of GM colony formation by human sera fractionated by column chromatography on Sephadex G-200. Normal serum (A) and chloroform extracted serum (B). (—) protein; (- - -) inhibitory activity for GM colony formation.