BIOSYNTHESIS OF COMPONENT C1q OF COMPLEMENT IN MOUSE PERITONEAL MACROPHAGE CULTURES

N. N. Kulagina, N. D. Ivanovska, I. A. Tarkhanova, and A. Ya. Kul'berg

UDC 612.112.95:612.118.223-083.337

KEY WORDS: component C1q of complement; mouse peritoneal macrophages.

An important function of macrophages in immune responses of the organism is their role in the synthesis of the components of complement and, in particular, C1q, C2, C3, C4, and C5 and factors B, D, and P [4, 9]. The great interest in the study of regulation of biosynthesis of C1q is due to the fact that this component, attached to the immune complex, initiates the triggering of the cascade of reactions of the classical complement fixation pathway.

Experiments *in vivo* have shown that immune complexes and their functional analogs lead to rapid intensification of C1q biosynthesis [1]. In experiments *in vitro*, biosynthesis of C1q by different cells (human and guinea pig peritoneal macrophages, fibroblasts, cells of cylindrical and transitional epithelium, blood monocytes) have been studied until recently mainly by the use of function tests [2, 6, 8, 10]. However, this does not permit a detailed analysis of the biosynthesis of a given protein and its comparison with the biosynthesis of other cell proteins. These opportunities are provided by the traditional isotope technique when combined with the method of radioimmunoassay.

In this investigation, biosynthesis of component C1q of complement was studied by radioimmunoassay in cultures of resident mouse peritoneal macrophages.

EXPERIMENTAL METHOD

Resident macrophages were obtained from the peritoneal cavity of (CBA × C57BL/6)F_1 mice weighing 18-20 g. The cells were introduced into siliconized centrifuge tubes and washed three times with medium 199 at 1000 rpm for 10 min in the cold. Medium 199 not containing glycine was then added and $2 \cdot 10^6$ cells in 1.5 ml of the same medium were introduced into siliconized penicillin flasks, on the bottom of which coverslips measuring 12 × 12 mm were placed. Incubation continued for 1 h at 37°C, after which the nonadherent cells were removed. The cultures were washed twice with warm medium 199 not containing glycine, after which they were treated with the same medium, with the addition of 200 mM glutamine, HEPES, and ^{14}C-glycine (1 μCi/ml). The cultures were fixed after 24, 48, 72, and 96 h. The culture medium was centrifuged and used to determine C1q and total protein.

The cells were removed from the coverslips by the method in [5], then disintegrated by freezing and thawing; the lysate was clarified by centrifugation and used to determine radioactivity of the intracellular C1q and total protein.

Labeled C1q was determined by affinity chromatography. Sepharose 4B, activated by BrCN, with immobilized IgG from rabbit serum against mouse C1q was used as the adsorbent.

To obtain serum against mouse C1q rabbits were immunized with mouse C1q, fixed to an immune precipitate (IP), obtained by the use of egg albumin and rabbit serum against egg albumin in equivalent proportions. Each rabbit was given an injection of 6 mg of IP, on which C1q from 5 ml of mouse plasma was fixed in the presence of 0.01 M Na_3-EDTA and 1% gordox. The complex of IP with C1q was suspended in 0.15 M NaCl and injected into the rabbit in Freund's complete adjuvant, in a volume of 0.3 ml, into the popliteal lymph nodes of the hind limbs and in a volume of 0.2 ml intradermally at several points in the dorsal region. The animals were reimmunized 1 month later with 1 ml of a suspension of the IP–C1q complex intravenously. The animal

N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR, N. P. Vershilova.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 101, No. 2, pp. 185-187, February, 1986. Original article submitted May 24, 1985.

0007-4888/86/0002-0213$12.50

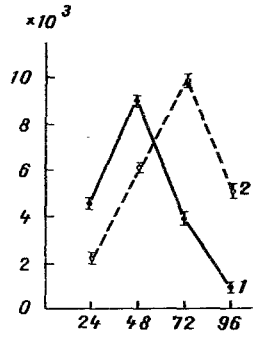

Fig. 1

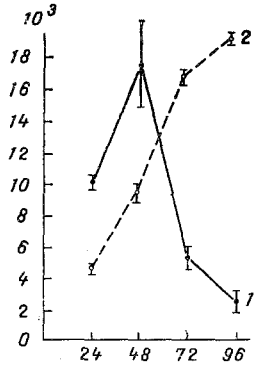

Fig. 2

Fig. 1. Synthesis of intracellular (1) and extracellular (2) Clq in cultures of resident mouse peritoneal macrophages. Abscissa, duration of culture (in h); ordinate, radioactivity of Clq, specifically bound with adsorbent and eluted from it (in cpm/2•10^6 explanted cells). 1) Intracellular Cl, 2) extracellular Cl.

Fig. 2. Synthesis of intracellular (1) and extracellular (2) protein in cultures of resident mouse peritoneal macrophages. Abscissa, duration of culture (in h); ordinate, radioactivity of protein (in cpm/2•10^6 explanted cells).

was bled on the 7th, 9th, and 11th days after reimmunization. The antiserum obtained was exhausted to remove contaminating antibodies against mouse immunoglobulins on the immunoglobulin fraction from mouse plasma, previously deprived of Clq [1], immobilized on Sepharose. The resulting mouse Clq were obtained by the method in [7]. Radioactive material was fixed on the adsorbent, eluted with concentrated formic acid, and applied in volumes of 0.2 ml of eluate to Whatman (England) filters, dried, and introduced into flasks with scintillation fluid. Radioactivity was measured by means of a Mark 2 liquid scintillation spectrometer. Total protein was determined as radioactivity of the samples precipitated with 5% TCA in a solution of bovine serum albumin (concentration 2 mg/ml) on Synpor filters.

Parallel cultures of resident mouse macrophages were set up on medium not containing ^{14}C-glycine. The cultures were fixed at the same times with 96% alcohol and stained with hematoxylin and eosin.

The significance of the results was estimated by the Student—Fisher t test. Differences were considered significant at the $P < 0.05$ level.

EXPERIMENTAL RESULTS

Morphologic analysis showed that cultures of resident mouse peritoneal macrophages were virtually free from contamination by other cells. The number of cells on a coverslip after 96 h in culture was virtually unchanged at $1.2 \cdot 10^5$ to $1.5 \cdot 10^5$. Single fibroblasts appeared only toward the 4th day of culture.

During culture of the macrophages in medium containing ^{14}C-glycine, radioactive protein, bound with immobilized antibodies against mouse Clq was found both in the culture fluid and in the cell lysate. If an excess of unlabeled mouse Clq, treated by the method described above, was added beforehand to the immobilized antibodies, the immunosorbent was no longer able to bind material from the culture fluid and lysate. Hence it follows that peritoneal macrophages do in fact synthesis Clq *in vitro* and secrete it into the external medium.

Maximal incorporation of label into Clq contained in the cell lysate occurred 48 h after the beginning of culture. The rate of Clq synthesis then decreased, so that after 96 h the cells contained virtually no Clq (Fig. 1). Parallel determination of radioactivity of all the intracellular proteins, and also of radioactivity of all proteins secreted by the cell, showed that in the first case the kinetics of their synthesis was the same as that for Clq, but in the second case an exponential increase in their content was observed (Fig. 2). Incidentally, Clq accounted for a considerable part of the total protein synthesized by macrophages.

Besides studying biosynthesis of Clq by resident mouse peritoneal macrophages, we also studied the biosynthesis of this protein in cells of macrophage-like line 6338-D_1. When these

cells were cultured under the same conditions as the resident macrophages, radioactive Clq was recorded in the culture fluid.

Differences observed in the kinetics of accumulation of radioactive Clq in the cells and in the external medium can evidently be explained on the grounds that after Clq formation *de novo* ceased, its continued accumulation in the external medium was due to release of protein from its intracellular depots.

The data of kinetic analysis of Clq biosynthesis by peritoneal macrophages, combined with the results of biosynthesis of total proteins by these cells suggest that biosynthesis of this protein is regulated by the feedback principle, and indeed that this process is regulated by Clq reaching the external medium. The signal is evidently transmitted into the cell through a specialized receptor for Clq, found *inter alia* on macrophages [3].

LITERATURE CITED

1. I. A. Tarkhanova, L. N. Chernousova, and A. Ya. Kul'berg, Immunologiya, No. 4, 16 (1984).
2. C. Bently, B. Zimmer, and U. Hadding, in: Lymphokines, E. Pick, ed., Vol. 4, New York (1981).
3. B. Ghebrehiwet, L. Silvestri, and G. McDevit, J. Exp. Med., 160, 1375 (1984).
4. H.-P. Hartung and U. Hadding, Agents Actions, 13, 415 (1983).
5. K. Kumagai, K. Itoh, S. Hinuma, et al., J. Immunol. Methods, 29, 17 (1979).
6. D. B. L. McClelland and R. van Furth, Immunology, 31, 855 (1976).
7. L. M. McManus and P. K. Nacane, J. Immunol. Methods, 36, 159 (1980).
8. K. M. Morris, H. R. Colten, and D. H. Bing, J. Exp. Med., 148, 1007 (1978).
9. D. E. Mosier, Science, 158, 1573 (1967).
10. K. B. M. Reid and E. Solomon, Biochem. J., 167, 647 (1977).

EFFECT OF MYELOPEPTIDES ON CYTOLOGICAL ACTIVITY OF T LYMPHOCYTES

I. G. Derevyanchenko, S. N. Bykovskaya, L. A. Zakharova, and S. V. Kibza

UDC 616.155.3-097.35.32

KEY WORDS: myelopeptides; T lymphocytes; immunity.

The immune response to viral and cellular antigens and to lectins is depressed in cancer patients, especially after treatment (by radiotherapy or chemotherapy), and the total lymphocyte count falls. The proportion of T suppressor cells and of Ia^+ cells is increased under these circumstances, although their total number also is reduced [7].

Furthermore, unlike the majority of tumors induced by chemical carcinogens or by oncogenic viruses, spontaneous tumors of man and animals are often nonimmunogenic [8].

The use of immunomodulators to activate the T and B systems of cellular immunity thus assumes great importance in cancer patients. Attempts are now being made to use preparations based on hormones and mediators of the immune system as immunocorrective agents. Thymus peptides, which ensure normal functioning of T lymphocytes [3], have become widely familiar.

On the basis of mediators of bone marrow origin (myelopeptides) the preparation known as myelopeptin has been produced. Its action is aimed at correcting the B system of immunity when the level of antibody production in the immune organism is depressed [5]. The possibility cannot be ruled out that myelopeptin may also be effective in certain disturbances of the T system of immunity, in view of evidence showing its interaction with individual subpopulations of T lymphocytes [2].

All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR. Institute of Immunology, Ministry of Health of the USSR, Moscow.

(Presented by Academician of the Academy of Medical Sciences of the USSR, R. V. Petrov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 101, No. 2, pp. 187-189, February, 1986. Original article submitted April 23, 1985.

0007-4888/86/0002-0215$12.50