MOUSE THYMOCYTE IMMUNOGLOBULIN : CAN PROTEOLYSIS EXPLAIN DIFFICULTIES IN ITS DETECTION ?

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SUMMARY. To examine if, as has been suggested, a peculiar proteolytic activity of thymus cell lysates might explain failures to detect immunoglobulin (Ig) biosynthesis by thymus cells, lysates of ^{14}C leucine labelled mouse myeloma cells were incubated with a 10^3 excess of unlabelled mouse thymus or spleen cell lysates, and then submitted to immune precipitation to isolate labelled Ig chains. Analysis of the immune precipitates by SDS polyacrylamide gel electrophoresis followed by radioautography failed to provide evidence for the purported proteolytic activity of the thymus cell lysates. Furthermore, thymus cell suspensions uncontaminated by plasma cells were biosynthetically labelled, then lysed in the presence or absence of Trasylol, an inhibitor of trypsin-like proteases. No labelled Ig chains could be detected under either condition of cell lysis. Evidence is presented that the detection of Ig chains synthesized by thymus cell suspensions might result from the contamination of these suspensions by plasma cells.

The nature of the specific antigen receptors on the cell membranes of thymocytes or thymus-derived (T) lymphocytes remains controversial. While Marchalonis et al (1) using cell surface radioiodination with the lactoperoxidase technique found significant amounts of IgM molecules on the surface of thymocytes, others, using surface radioiodination (2,3,4) or biosynthetic labelling (4) , have been unable to detect IgM on various populations of thymocytes or T lymphocytes under conditions where detection of B lymphocyte is straightforward (4). Recently, however, Moroz and Hahn have suggested that these discrepancies might result from the presence in thymus extracts of a strong proteolytic activity towards Ig chains (5). They reported that as much as 25 to 30 percent of the protein synthesized in

short term cultures of human thymocytes could be identified as μ and L Ig chains, provided that thymocyte homogenization was performed in the presence of an inhibitor of trypsin-like proteases (Trasylol). Using the same precautions, Ig synthesis by mouse thymus cells was also detected, but the class of the Ig varied with the mouse strain, Balb/c mice synthesizing IgA and C57/Bl mice IgM molecules (5). The present work explores the possibility that a special proteolytic activity of mouse thymus cell lysates might explain previous failures to detect Ig chains in radioiodinated or biosynthetically labelled mouse thymus cells.

MATERIALS AND METHODS. Thymic cell suspensions of (C57/Bl x CBA) F_1 mice were treated in the presence of complement with rabbit antisera against mouse B lymphocytes and mouse plasma cells as in previous experiments (4), to kill contaminating non thymus-derived cells. Thymocytes, or cell suspensions of myelomas MOPC 104 E (an IgM synthesizing tumor) or MOPC 41 (a k chain synthesizing tumor) were incubated for 4 hrs at 37° with either ^{35}S methionine for thymocytes (15 μC/ml, Amersham, 140 C/mmol), or ^{14}C leucine for myeloma cells (2 μC/ml, New England Nuclear, 327 C/mol) (4). At the end of the incubation, the cells were washed and homogenized in buffer containing 0.5% NP_{40} (Shell Oil Co.) with or without Trasylol (Bayer) as described in the text. The homogenates were spun for 60 min at 100.000 g max, and the supernatants (cell lysates) submitted to specific immune precipitation to isolate labelled Ig chains, or to control non specific immune precipitation, as described in detail elsewhere (4). The precipitates were washed, solubilized, reduced in SDS containing buffer and submitted to SDS polyacrylamide gel electrophoresis followed by radioautography (4). Immunofluorescent staining was performed either on thymus frozen sections or on smears of thymic cell suspensions obtained from Balb/c and C57/Bl mice, using rhodamine labelled rabbit antisera directed against mouse Ig, or specific for mouse μ or α chains (6).

RESULTS AND DISCUSSION. The cell lysates obtained from 10^5 myeloma cells labelled with ^{14}C leucine were mixed with the cell lysates obtained from 1.5×10^8 normal thymus or spleen cells, or with 0.5 ml of phosphate-buffered saline (PBS) as a control. These mixtures were left for 15 to 60 min at room temperature in order to facilitate any proteolytic activity present in the tissue extracts, and then submitted to immune precipitation to isolate labelled Ig chains. The immune precipitates contained the following percentages of the total acid precipitable radioactivity added to the various mixtures: 1) MOPC 104 lysate mixed with thymus cell lysate : 11.5 %; with spleen cell

lysate : 15.6 %; with PBS : 13.1 %; 2) MOPC 41 lysate mixed with thymus cell lysate : 9.3 %; with spleen cell lysate : 9.3 %; with PBS : 9.2 %. Radioautographic analysis of the precipitates (fig. 1) showed the expected bands

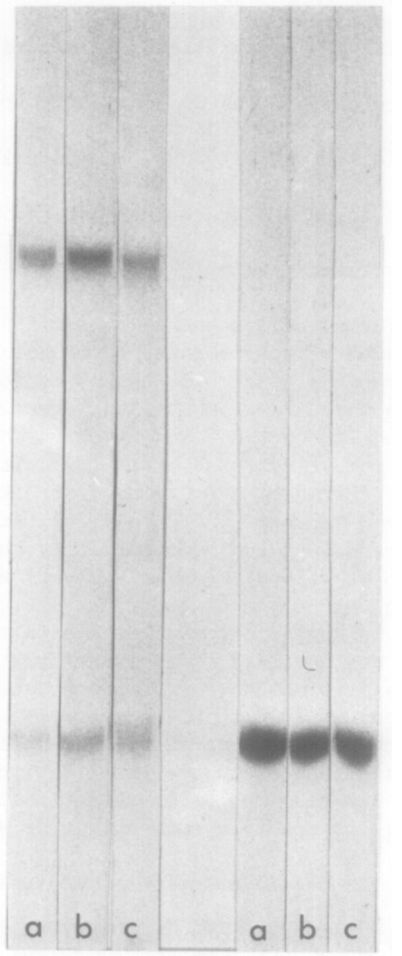

Figure 1. Radioautographs of SDS polyacrylamide gels of the reduced immune precipitates obtained from lysates of ^{14}C leucine labelled MOPC 104 E cells (I), and MOPC 41 cells (II) incubated in presence of: a) phosphate-buffered saline, b) thymus cell lysate, c) spleen cell lysate.

of labelled Ig chains, whose intensity was similar in precipitates obtained from thymus cell lysate, from spleen cell lysate or from PBS; there were no detectable radioactive bands which might correspond to partially degraded μ or L chain. Thus, these experiments failed to provide evidence for a

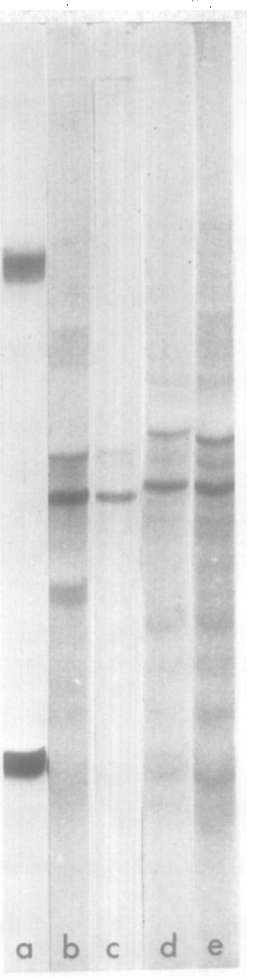

Figure 2. Radioautographs of SDS polyacrylamide gels of reduced immune precipitates :
a) Specific precipitates of the incubation medium of MOPC 104 E cells labelled in vitro with ^{14}C leucine showing the position of secreted μ and L chains
b-c) Specific (b) and control non specific (c) precipitates from ^{35}S methionine labelled thymus cells lysed in the presence of Trasylol
d-e) Specific (d) and control non specific (e) precipitates from ^{35}S methionine labelled thymus cells lysed in absence of Trasylol.

proteolytic activity of the thymus or spleen extracts towards μ or L Ig chains.

It remained possible, however, that thymocytes make Ig chains of a special type, which might be unusually sensitive to a proteolytic activity present in thymus extracts. To explore this possibility, thymocytes were incubated for 4 hrs with ^{35}S methionine, washed, and divided into two parts. The

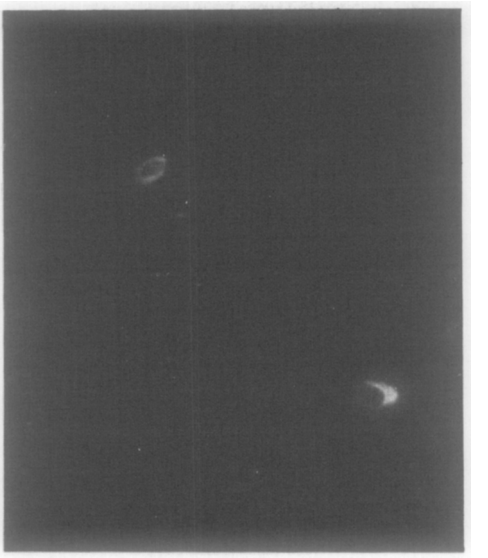

Figure 3. Frozen section from a Balb/c thymus stained with a rhodamine labelled rabbit anti mouse α chains antiserum. Two IgA containing plasma cells are seen in this field.

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first part was homogenized in 0.5 % NP_{40}, and the second in 0.5 % NP_{40} containing Trasylol (50 Kiu/ml) and 0.2 M iodoacetamide, i.e. under the conditions used by Moroz and Hahn (5). The cell lysates were then submitted to immune precipitation, and for the sample homogenized in the presence of Trasylol, all the subsequent steps were performed in solutions containing Trasylol and iodoacetamide. The cell lysate of the Trasylol-treated samples contained 30% more acid precipitable radioactivity than the cell lysate obtained in the absence of Trasylol. However, analysis by SDS gel electrophoresis and radioautography of the specific and control immune precipitates, which contained from 1 to 4% of the total acid precipitable radioactivity, showed identical results in the samples homogenized in the presence or absence of Trasylol (fig. 2). As was found in previous experiments (4), no radioactive bands corresponding to Ig chains were observed, but only bands representing labelled chains apparently unspecifically trapped in the immune precipitates, since they were similar in the control and specific

precipitates. Thus, these experiments failed to provide evidence that thymus cell suspensions, uncontaminated by plasma cells and B lymphocytes, synthesize Ig chains which are detected only when the cell homogenization is performed in the presence of an antiproteolytic agent.

Immunofluorescent studies on thymus sections or smears of thymus cell suspensions revealed that plasma cells are not rare in the thymus of Balb/c and C57/Bl strains, the majority of these plasma cells containing IgA in Balb/c mice (fig. 3) and IgM in C57/Bl mice. The finding that IgA and IgM molecules are precisely those which were found to be synthesized by the thymus cell suspensions of Balb/c and C57/Bl strains respectively (5) suggest the possibility that these detected Ig molecules might come from a small number of contaminating plasma cells (7).

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REFERENCES

1. Marchalonis, J.J., Cone, R.E. and Atwell, J.L. (1972). Proc. Nat. Acad. Sci. USA 69, 2556-2560.
2. Vitetta, E.S., Bianco, A., Nussenzweig, V. and Uhr, J.W. (1972). J. Exp. Med. 136, 81-93.
3. Grey, H.M., Kubo, R.T. and Cerottini, J.C. (1972). J. Exp. Med. 136, 1323-1328.
4. Lisowska-Bernstein, B., Rinuy, A. and Vassalli, P. (1973). Proc. Nat. Acad. Sci. USA 70, 2879-2883.
5. Moroz, C., Hahn, Y. (1973). Proc. Nat. Acad. Sci. USA 70, 3716-3720.
6. Lamelin, J.P., Lisowska-Bernstein, B., Matter, A., Ryser, J.E. and Vassalli, P. (1972). J. Exp. Med. 136, 984-1007.
7. Vitetta, E.S., Uhr, J.W. and Boyse, E.A. (1973). Proc. Nat. Acad. Sci. USA 70, 834-838.