cells labeled with ^{3}H-thymidine, between 48 and 72 h, to be greater in LIF pretreated cells than in controls. To this end cells grown for 48 h with or without LIF were newly diluted and placed in a new medium containing ^{3}H-thymidine. As predicted by the 2nd hypothesis the percentage of labeled cells is higher in LIF treated cells with respect to the control (table).

The mechanisms by which the cells are prevented from entering the S phase are still unknown. It is worth noting that the antiproliferative effect appears with a latency of at least 24 h. Since this latency cannot be attributed to a low membrane permeability to LIF, as shown by the fact that the restoration of the proliferative activity follows immediately upon LIF removal, a time consuming process must be involved, the nature of which remains to be determined. As shown by Pietu et al.[3], LIF inhibits DNA synthesis by preventing the increase in DNA polymerase. It may be speculated that a similar action could be responsible for the effect we have observed on erythroleukemic cells.

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0014-4754/83/020180-03$1.50 + 0.20/0

Monoclonal antibodies to metallothionein from Cd^{2+}-resistant Chinese hamster lung fibroblasts

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Summary. Four monoclonal antibodies of the mouse against metallothioneins (MTs) from Cd^{2+}-resistant fibroblasts of the Chinese hamster lung (Cd^r-CHL) have been prepared. Each one of the antibodies showed a unique cross-reactivity pattern when tested against MTs from the livers of several mammals and from yeast.

Metallothionein (MT), a low molecular weight protein with a high content of Cd, does not contain aromatic amino acids, and about 1/3 of its amino acid content is cysteine. Detailed analysis of MTs revealed that 2 components (MT-1 and MT-2) of similar amino acid composition and molecular weight are produced (for a review, see Nordberg and Kojima[2]). An immunoassay for MT offered great advantages in terms of specificity, sensitivity, and precision, but the conventional antisera obtained against MTs show cross-reactivity between the 2 molecular species and among MTs derived from various animals[3-8]. Monoclonal antibodies against each component of MTs should overcome these disadvantages of conventional antisera[9]. In this paper, we report the establishment of hybridoma lines producing monoclonal antibodies reactive to MTs, and describe their reactivities to MTs of several mammals and yeast.

Materials and methods. Chinese hamster lung fibroblastic cells made resistant to 50 μM or 100 μM Cd^{2+} (50 μM or 100 μM Cd^r-CHL) were used as the source of MTs. The cells were made resistant to $CdCl_2$ by increasing the concentration of $CdCl_2$ in the culture medium stepwise, beginning from 5 μM, at intervals of 1–2 weeks. Heat-stable cytoplasmic fraction was applied to Sephadex G-75 and subsequently to a DEAE-Sephadex column[4]. The 2 resulting peaks of Cd were separately pooled and lyophilized. Fusion of the spleen cells of a BALB/c mouse immunized with MT-2 of Cd^r-CHL and NS-1 cells was carried out as described by Oi and Herzenberg[10]. Hybridomas were selected by ELISA using MT-1 and MT-2 of Cd^r-CHL as antigens[11]. Immunoglobulin subclass was determined by the double diffusion method[12]. Rabbit antisera against purified mouse IgA, IgG_1, IgG_{2a}, IgG_{2b}, IgG_3, and IgM were purchased from Miles Laboratory. Interspecies cross-reactivity of monoclonal antibodies was examined by ELISA using various MTs as antigens.

Results and discussion. Almost 90% of Cd of the heat-stable supernatant of 100 μM Cd^r-CHL was associated with a low molecular weight peak exhibiting high absorption at 254 nm with low absorbance at 280 nm (fig., a). This MT fraction was applied to a DEAE-Sephadex column and separated into MT-1 and MT-2 (fig., b). The elution pattern of heat-stable cytoplasmic supernatant of 50 μM Cd^r-CHL was not significantly different from that of 100 μM Cd^r-CHL. Control CHL cells gave no MT peak in the Sephadex G-75 elution pattern. Lyophilized preparation of MT-1 and MT-2 each showed a single band in polyacrylamide slab gel electrophoresis. Preliminary amino acid

Immunoglobulin subclass and interspecies cross-reactivity pattern of monoclonal antibodies

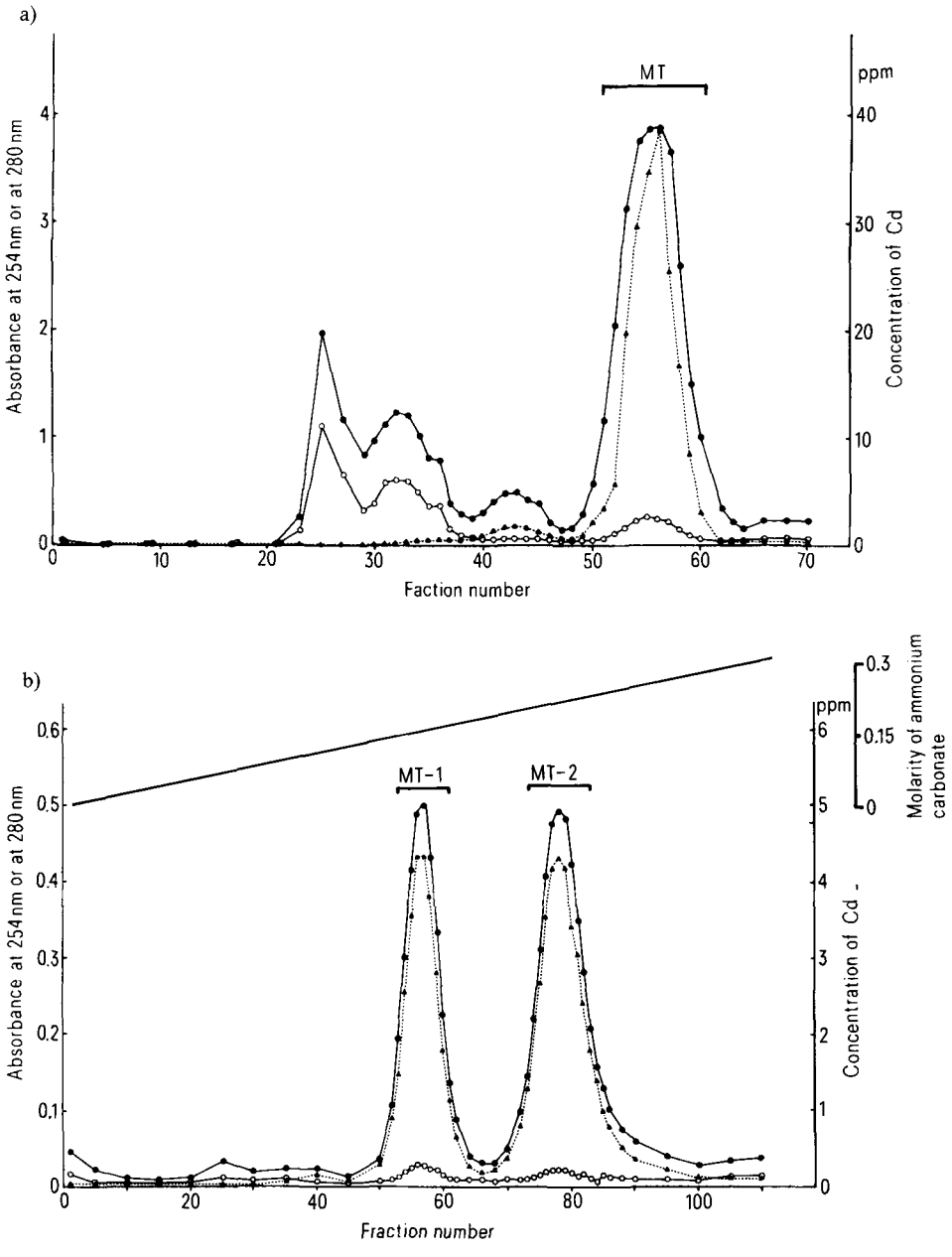
Subclass	ACM-1 IgM	ACM-2 IgG_{2b}	ACM-3 IgM	ACM-4 IgG_{2b}
Cd^r-CHL MT-1	+	−	±	−
2	+	+	+	+
Mouse L MT-1	+	−	±	−
2	+	+	+	±
Rabbit L MT-1	+	+	+	−
2	+	+	+	−
Pig L MT	+	+	+	−
Bovine L MT	+	−	−	−
Monkey L MT-I	+	+	+	−
II	+	+	+	−
III	+	+	+	−
IV	+	+	+	−
V	+	+	+	−
Yeast MT	−	−	−	−

Preparation of MT: Cd^r-CHL MT-1 and 2, see 'Materials and methods'. Mouse L-MT-1 and 2[13]. Rabbit L-MT-1 and 2[14]. Monkey L-MT-I, II, III, IV and V[15]. Yeast MT[16]. Pig L-MT; the MT fraction on Sephadex G-75 gel filtration was prepared from the liver of Cd-treated pig according to the method described by Kimura et al.[14]. Bovine L-MT: the MT was prepared from the bovine liver (unpublished). Abbreviation: L; liver.

analysis of MTs of Cd^r-CHL revealed that the MTs contained no aromatic amino acid residue, and that 30% of the amino acid residues (molar ratio) were cysteine. These results show that this protein belongs to the 'metallothionein' category[2]. At the same time, this finding demonstrates that the fibroblastic cells of the Chinese hamster can synthesize 2 molecular species of MT in vitro in response to Cd^{2+}.

Four clones of hybridomas secreting anti-MT antibodies were obtained. Of the 4 monoclonal antibodies examined, each one had a distinct specificity pattern in reacting with MTs of several mammals and yeast (for these MTs refer to the legend to the table). Of these, the one designated as ACM-4 is the most specific, and ACM-1 appears to be least specific. Antibodies of ACM-2 and ACM-3 may appear to be fairly similar in reactivity, but the subclass of immunoglobulin is different in these 2 antibodies.

In radioimmunoassay of MT, rat liver MT was employed as an antigen for immunization of rabbits, but the conventional antisera could not distinguish between the molecular species[4-6], or rat, rabbit, equine, human, and Chinese hamster culture cell MTs[5,6,8]. In the present study it was shown that each of the monoclonal antibodies examined showed a unique specificity pattern in reacting with MTs. Mouse liver MTs immunologically resemble MTs of Cd^r-CHL. The mouse hybridomas secreted antibodies which bound to their own MTs. The significance of this autoimmunity-like phenomenon remains to be determined. On the other hand, the monoclonal antibodies showed similar patterns of cross-reactivity against liver MTs of rabbit, pig, and monkey. Bovine liver MT has a unique reactivity, and yeast MT did not react with our monoclonal antibodies. The positive well of each antigen provided confirmation of coating by antigens. Thus, low adsorbing efficiency of plastic wells for yeast MT cannot be ruled out. We obtained no clone secreting MT-1 specific antibody, probably because we started with hybridoma from spleen cells of an MT-2 immunized mouse. Even so, these



a Chromatography of the heat-stable supernatant of 100 μM Cd^r-CHL on a 2.6 × 70 cm column of Sephadex G-75, eluted with 0.01 M ammonium formate, pH 7.4, at a flow rate of 25 ml/h. Cadmium (▲----▲), and absorbances at 245 nm (●—●) and 280 nm (○——○) were measured. The MT fraction is indicated by the bar. *b* Chromatograph of MT fraction from figure *a* on a DEAE-Sephadex column (1.6 × 30 cm). Linear gradient elution (0.01-0.3 M ammonium carbonate buffer pH 8.6) was carried out at a flow rate of 50 ml/h. Two MT peaks were eluted and numbered as MT-1 and MT-2.

4 monoclonal antibodies make it possible to discriminate MT-1 and MT-2 of Cd^r-CHL and of mouse liver. Thus, these antibodies represent a set of reagents with high specificity for use in biochemical and biological studies of MTs.

1 This work was supported in part by a Grant-in-Aid for Cancer Research, the Ministry of Education, Science and Culture. We wish to thank to Dr T. Akatsuka, Dept. Genetics, Institute of Medical Science, University of Tokyo, for advice and for providing NS-1 cells, and Dr S. Tsukagoshi, Cancer Chemotherapy Center, for providing protein isolation facilities. The skillful technical assistance of Mrs K. Nemoto and Mrs N. Otaki is gratefully acknowledged.

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0014-4754/83/020182-03$1.50 + 0.20/0

Side effects of *Ricinus* lectin (RCA 120) on nucleic acid synthesis in chick embryo fibroblasts

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Summary. When fibroblasts from chick embryos were treated with *Ricinus* lectin, the effects observed depended on the stage of development of the embryo from which the cells were prepared. Thus, in 16-day fibroblasts, which have a weak proliferative capacity, nucleic acid synthesis was less sensitive to the effect of this lectin than that in 8-day fibroblasts, whose proliferative capacity is high.

Ricinus lectin (RCA 120) has been found to have a toxic effect on protein synthesis[3-6]. Only a few reports, however, have described its side effects on nucleic acid synthesis[7,8]. We previously have shown in chick embryo fibroblasts that the toxic effect of *Ricinus* lectin, as estimated by leucine incorporation, was differential during the embryo development[9]; thus, cells from young embryos were less sensitive than those from older ones, and the younger cells proliferated faster. It was therefore of interest to investigate the possible differential effect of Ricinus lectin on nucleic acid synthesis.

The present study describes this lectin's effects on such synthesis in chick embryo fibroblasts from 2 stages of embryonic development.

Materials and methods. Cells. Fibroblasts were obtained from 8- and 16-day chick embryos as previously described[10]. *Ricinus* lectin, the tetrameric form of *Ricinus communis* lectin (mol.wt 120,000) was purified by the procedure of Nicolson and Blaustein[11]. The lectin solution was prepared at a final concentration of 100 µg/ml in 0.15 M NaCl and was added to cell cultures at final concentrations ranging from 0.001 to 10 µg/ml. As to cell cultures, primary monolayers were made in 16 mm diameter wells using 0.5 ml of Eagle's medium (MEM, Flow) supplemented with 1% glutamine, 1% antibiotics (penicillin and streptomycin) and 10% foetal calf serum. The initial seeding concentration was 10^6 cells/ml. Cultures were grown in humidified air with 5% CO_2. A sample of cells was counted in a hemocytometer. Each measurement refers only to viable cells and represents the mean of 4 samples. For estimating nucleic acid synthesis, cell cultures were incubated for 1 h at 37 °C with 0.5 µCi of tritiated thymidine or uridine per well (Radiochemical Centre Amersham, sp. act.: 26 and 25 Ci/mmole, respectively). The cells were then removed from the wells by trypsin treatment as previously described[12]. The labeled cellular material was then allowed to precipitate overnight in 2 ml of trichloroacetic acid (TCA) 10% (v/v). The precipitate was washed twice with 5% TCA (v/v), dissolved in 3 ml ACS (Searle, Amersham) and radioactivity was then measured using a liquid scintillation spectrometer (Intertechnique SL 30).

Results and discussion. The fibroblasts from 8- and 16-day embryos reached subconfluency after 48 and 96 h respectively. *Ricinus* lectin was added to subconfluent cultures and its effects were noted after various incubation times. The number of cells ($0H.95 \pm 0.07 \times 10^6$ cells/well) and cellular protein content (147 ± 25 µg/10^6 cells) estimated by the method of Lowry et al.[13], remained unchanged in both control and *Ricinus* lectin-treated cultures from older and younger embryos.

As figures 1,a and b show, the optimum concentration of *Ricinus* lectin was 1.5 µg/well in cells from both types of embryo. This concentration was therefore used to study the time course of the effect of lectin (table). After 2 h incubation with *Ricinus* lectin, ^{3}H-thymidine incorporation was inhibited by 64 and 34% in 8- and 16-day cells respectively. The same incorporation was totally inhibited after 4 h of incubation in 8-day cells, but only partly