Studies on J-Chain Biosynthesis in Tumours Producing Immunoglobulins in NZB Mice

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Investigation of biosynthesis of J chain in plasmacytomas induced in NZB mice revealed that this protein was not only synthesized in the cells that produce polymer immunoglobulin A but also in those that produce immunoglobulin G monomer. It was also found that protein similar to J chain of BALB/c-mice was associated with polymer immunoglobulin A but not with immunoglobulin G of NZB mouse myeloma proteins.

The monomeric immunoglobulin molecules of all classes consist of two light (L) chains and two heavy (H) chains, whereas polymeric immunoglobulins are made up of two or more subunits joined together with a J chain (Halpern & Koshland, 1970). J chain is synthesized even in plasmacytoma cells that produce only monomer immunoglobulins (IgG* class) (Kaji & Parkhouse, 1974), and production of J chain is not tightly coupled to heavy-chain production (Kaji & Parkhouse, 1975). Previous investigations (Abel & Grey, 1968) have shown that IgA immunoglobulins derived from the BALB/c strain of mice do not have a disulphide bridge between the light and heavy chains, in contrast with human IgA molecules of IgA₁ class and IgA₂-Am negative allotype group and IgA synthesized in NZB mice plasmacytomas, in which heavy and light chains are linked by disulphide bridges (Warner & Marchalonis, 1972). Because of the presence of disulphide bonds between L and H chains of NZB mouse IgA proteins one might expect that IgA and J-chain molecules produced in these mice may be different from the IgA and J-chain molecules produced by BALB/c strain. If this is indeed the case, we can investigate whether the structural gene for J chain is linked to that for H chain by analysing the J chain in recombinant inbred strains. In the present paper I report that protein similar to J chain was found in IgA molecules of NZB mouse myeloma proteins, and that synthesis of J chain not only took place in the cells that produce polymer IgA but also in those that produce IgG monomer.

Materials and Methods

Plasmacytomas were induced in NZB mice by Dr. M. Weigert and Dr. R. Riblet at the Institute for Cancer Research. Immunoprecipitation (Crowle, 1960) and immunodiffusion assays with 1.5% (w/v) Ionagar in barbital buffer at pH 8.6 were performed

on sera of tumour-bearing mice against rabbit antisera of various classes of immunoglobulins, to identify types of immunoglobulins produced by these mice.

Analysis of J-chain synthesis

For the assay of J-chain synthesis tumour cells (1×10^8 – 2×10^8) were incubated at 35°C with 200–500 μ Ci of L-[4,5-³H]leucine (30 Ci/mmol) (New England Nuclear Corp., Boston, MA, U.S.A.) for 1.5–4 h in 5 ml of Dulbecco modified Eagles' medium (minus leucine). After incubation, the cells were collected by centrifugation (500 g for 5 min) and lysed in 2 ml of a solution containing 1% (w/v) Nonidet P.40 and 100 mM-iodoacetamide in phosphate-buffered saline (130 mM-NaCl/10 mM-sodium phosphate, pH 7.4). The lysate was centrifuged at 30000 g for 30 min. A 1 ml portion of supernatant was caused to react with 15 μ l of rabbit anti-(J chain) serum for 30 min at 37°C; goat anti-(rabbit IgG) serum was then added, and incubation was continued for 1 h at 37°C. The precipitate thus obtained was collected by centrifugation (5000 g for 10 min) and washed with 3 $\times$ 2 ml of phosphate-buffered saline containing 0.5% (w/v) Nonidet P.40. The washed precipitate was suspended in 0.1 ml of 16 mM-Tris/50 mM-glycine, pH 8.7, containing 9 M-urea and 10 mM-dithiothreitol, and incubated for 1 h at 37°C. The reduced precipitate was alkylated by adding iodoacetamide (20 mM final concn. at 26°C for 30 min) and was electrophoresed in a 0.6 cm $\times$ 8 cm tube at 3 mA/tube, as described by Reisfeld & Small (1966) in 'alkaline urea gels', with NN'-diallyltartardiamide (Aldrich Chemical Co., Milwaukee, WI, U.S.A.) as a cross-linking agent in place of methylenebisacrylamide (Anker, 1970). After electrophoresis, gels were sliced into 1 mm widths, dissolved in 2% (w/v) HIO₄, and radioactivity was measured in a toluene/Triton X-100 (2:1, v/v) scintillation mixture. The radioactivity appearing in the J-chain peak was expressed as the percentage of the total trichloroacetic acid-insoluble radioactivity.

* Abbreviations: IgG, IgA, immunoglobulin G, immunoglobulin A.

Analysis of immunoglobulin from incubation of plasmacytoma and tumour cells

Radioactive supernatant containing secreted immunoglobulins was subjected to sodium dodecyl sulphate/polyacrylamide-gel electrophoresis to determine the immunoglobulin classes of the products. Supernatant, free of cells, was caused to react with anti-(rabbit IgA), anti-(rabbit IgG), or anti-(rabbit IgM) sera and then precipitated with purified proteins IgA, IgG or IgM respectively. Precipitated protein was washed with 3×2ml of phosphate-buffered saline, containing 0.5% (w/v) Nonidet P.40, and then suspended in 0.1ml of 0.02M-phosphate buffer, pH7.0, containing 2mg of each of sodium dodecyl sulphate and iodoacetamide and boiled for 5min. Solubilized materials thus obtained were electrophoresed at 15mA/tube (0.6cm×8cm) for 3.5h on sodium dodecyl sulphate/polyacrylamide gel (Summers *et al.*, 1965). Electrophoresed gel was sliced, dissolved and counted for radioactivity as for analysis with alkaline urea gels.

Purification of immunoglobulins

Serum (7ml) obtained from tumour-bearing mice was diluted with an equal volume of phosphate-buffered saline, and $(\text{NH}_4)_2\text{SO}_4$ was added to a final concentration of 50%. The precipitate was dissolved in 13ml of phosphate-buffered saline, dialysed against 0.01M-Tris/HCl, pH8.0, for 16h, and placed on a column (2.5cm×20cm) of DEAE-cellulose DE 52. Chromatography was carried out with a linear 500ml gradient containing 0–0.25M-NaCl in 0.01M-Tris/HCl, pH8.0. Fractions were tested for immunoglobulin by agar-gel electrophoresis, and appropriate fractions were pooled. The immunoglobulin pool was concentrated by ultrafiltration through an Amicon XM50 membrane (Amicon Corp., Lexington, MA, U.S.A.). The protein solution (97.2mg) was dialysed against a solution containing 0.15M-Tris/HCl, pH8.0, 0.15M-NaCl and 0.002M-EDTA overnight. It was reduced with 0.01M-dithiothreitol for 1.5h at 37°C and alkylated with 0.022M-iodoacetate at 4°C for 30min. Reduced and alkylated protein was dialysed against 4M-urea in 1M-propionic acid for 3h and then fractionated by gel filtration on a column (2.5cm×1000cm) of Sephadex G-150 in the same urea/propionic acid solvent.

Protein was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as standard.

Results

Identification of immunoglobulin classes of various plasmacytomas and tumour 141

Immunoglobulin classes of tumours were first determined by testing sera of tumour-bearing mice by agar-gel immunodiffusion against anti-IgM,

-IgA or -IgG sera obtained from rabbits. Since it was not necessary to identify subclasses of immunoglobulins of the IgG-producing cells for the present study, classification of these tumours was not attempted. To ascertain classes of the radioactive immunoglobulins produced by plasmacytomas, labelled protein was prepared from the various tumour cells and precipitated with rabbit anti-serum specific for IgG, IgA or IgM together with homologous pure immunoglobulin. The precipitate was then subjected to sodium dodecyl sulphate/polyacrylamide-gel electrophoresis, and immunoglobulin classes of each product were identified from the position of radioactive proteins, as shown in Fig. 1. It is clear from Fig. 1 that the polymer produced by tumour 141 migrated more slowly than that produced by the other tumours, indicating it to be IgM. Immunoglobulin synthesized by tumour 938 behaved as a monomer IgG, and that of tumour 270 was IgA. These data were consistent with the results obtained by agar-gel immunodiffusion tests.

Fig. 1. Analysis of product synthesized by NZB mouse plasmacytomas and tumour 141

Tumour cells were incubated with L-[4,5- ^3H]leucine for 4h, and supernatant, free of cells, was obtained by centrifugation. Radioactive supernatant, containing 2.78×10^4 , 8.02×10^4 and 1.97×10^5 c.p.m. of tumours 141(a), 270(b), 938(c) respectively, was caused to react with rabbit antisera against IgM (MOPC 104E), IgA (MOPC 315) and IgG $_{2a}$ (5563), and precipitated by the addition of purified homologous proteins. The precipitate was washed with 3×2ml of phosphate-buffered saline containing 0.5% Nonidet P.40 and electrophoresed on sodium dodecyl sulphate/polyacrylamide gel. Gels were sliced, and radioactivity recovered in each slice was counted and plotted.

EXPLANATION OF PLATE I

Release of J chain from IgA produced by NZB mouse plasmacytomas

Purified immunoglobulin (0.7 mg) was suspended in 0.1 ml of Tris/glycine buffer containing 9 M-urea with or without dithiothreitol, alkylated and electrophoresed in alkaline urea gel. The right-hand-side gel in each immunoglobulin species contained immunoglobulin that had not been reduced with thiothreitol, whereas those on the left show the electrophoretic patterns of reduced and alkylated proteins. As a control, 50 μ g of purified mouse J chain was electrophoresed.

Table 1. *Presence of intracellular J chain in NZB mouse plasmacytomas and NZW mouse tumour*

For details see the text.

Tumour	Product	$10^{-2} \times$ J chain (% of total radioactivity)
141	IgM	1.2
270	IgA	3.66
599	IgA	3.57
1047	IgG	1.91
199	IgG	2.01
938	IgG	4.35
657	IgG	5.65

Presence of J chain in IgA synthesized and secreted by NZB mouse plasmacytoma cells

J chain was found only associated with polymeric immunoglobulin. It was therefore considered that the function of J chain was the joining of monomers (Halpern & Koshland, 1970). To see if this was also true for NZB mice, whose immunoglobulin has an unusual structure, purified immunoglobulin from these mice was electrophoresed in alkaline urea gel with or without reduction. As shown in Plate 1, a protein migrating faster than most of the other proteins appeared only when the immunoglobulins from tumours 373 and 2573 (both IgA type) were reduced with dithiothreitol. Although the electrophoretic mobility of this fast-migrating protein was broader, it was similar to that of the purified authentic J chain and J chain released from tumour MOPC-315 protein reduced with dithiothreitol. The fact that J chain appeared only on reduction strengthens the view that this component is unrelated to other cellular contaminants. Absence of the fast-migrating band from reduced alkylated proteins of tumours (280 and 920) producing IgG indicates that J chain is only found in the polymeric form of immunoglobulin, and not in monomeric immunoglobulin.

Presence of intracellular J chain in IgG-, IgA- and IgM-producing plasmacytoma and tumour cells

The previous finding that intracellular J chain can be detected (Kaji & Parkhouse, 1974) in monomer IgG-producing strain C3H and BALB/c mouse plasmacytomas suggests that synthesis of J chain may not be turned off. Because of the structural difference between immunoglobulins produced by NZB and BALB/c mice, the question of J-chain synthesis in relation to synthesis of other immunoglobulins was examined. In this experiment (Table 1) tumour cells from NZB mice were allowed to incorporate amino acid in cell suspension. Radioactive cellular protein was first caused to react with anti-(J chain) serum

Fig. 2. *Identification of J chain in cell lysate of NZB 920 mouse myeloma cells*

(a) Cell lysate labelled with L-[4,5- 3 H]leucine was caused to react with rabbit anti-(J chain) sera and precipitated with goat anti-(rabbit IgG). The washed precipitate was electrophoresed in alkaline urea gel, and radioactivity in the sliced gel was plotted against gel fraction. (b) Same as above except rabbit anti-(J chain) serum was absorbed with $2 \mu\text{g}$ of mouse J chain before reacting with total cell lysate.

and then precipitated with goat anti-(rabbit IgG) serum, and the precipitate was subjected to polyacrylamide-gel electrophoresis. Radioactivity corresponding to J chain was measured and expressed as a percentage of the total trichloroacetic acid-insoluble material. It is noted in Table 1 that all tumour lines, including monomer producers such as 657, 199, 1047 and 938, produced significant amounts of J chain. When the anti-(J chain) serum used in this experiment was first absorbed with purified mouse J chain no appreciable radioactivity appeared at the position of J chain during electrophoresis on alkaline urea gel (Fig. 2), thus establishing that the peak radioactivity is indeed due to J chain. These intracellular J chains were not associated with immunoglobulin and existed as a free form (Kaji & Parkhouse, 1974). In a separate experiment (results not shown) free J chain could not be found in the secreted material, even after prolonged (4–5 h) incubation with radioactive amino acid.

Discussion

IgA immunoglobulins of NZB mice have a disulphide bridge between their L and H chains (Warner & Marchalonis, 1972). The disulphide bond between L and H chains is found in human IgA molecules of IgA₁ class and IgA₂-Am negative allotype group (Warner & Marchalonis, 1972). Its function is not

clear, because properly functioning IgA of other strains of mice such as BALB/c do not have this bond. It was decided to examine the synthesis of J chain in tumour lines derived from NZB mice, to see if the presence of the disulphide bridge influences the control of J-chain biosynthesis in plasma cells. The results suggest that J chain was synthesized in NZB mouse plasmacytoma in a fashion similar to that in other strains of mice that do not possess the disulphide bridge between L and H chains. A polypeptide similar to J chain was found to be associated only with the polymeric form of immunoglobulin and therefore appears to act by joining subunits together, as originally suggested (Halpern & Koshland, 1970). However, J chain released from IgA produced by NZB mouse plasmacytomas showed broader peaks, suggesting possible chemical differences in J-chain structure between NZB and MOPC mouse plasmacytomas. The biosynthesis of J chain can be observed even in the monomer IgG-producers of NZB myeloma cells 199, 1047, 657 and 938. These data are consistent with the previous observation that synthesis of J chain is not obligatorily coupled to its function (Kaji & Parkhouse, 1974). J chain was also found to be synthesized in the IgM-producing tumour 141 derived from NZW mice. This observation suggests that the production of J chain does not depend on the specific cell types induced by neoplastic agents, such as mineral oil.

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