ULTRASTRUCTURAL STUDIES OF HUMAN LYMPHOID CELLS

μ and J Chain Expression as a Function of B Cell Differentiation*

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J chain expression in lymphoid cells has been detected by a variety of immunohistochemical techniques (for reviews see 1–4). Studies of mouse plasmacytomas initially suggested that J chain was synthesized only in cell lines that produced polymeric immunoglobulins (5, 6). This result supported the idea of J chain involvement in the process of immunoglobulin polymerization (2, 7, 8). However, subsequent studies have revealed that J chain may also be present in cells that contain monomeric immunoglobulins, heavy chains, light chains, or no immunoglobulin chains (3, 4, 9–15). The latter finding is particularly intriguing because it suggests that J chain expression may occur independently of immunoglobulin synthesis.

With respect to B cell maturation, Mather et al. (16) concluded that J chain synthesis is initiated during the switch from synthesis of membrane IgM to secretory IgM. In these studies of mouse lymphoid cells, early B cells and splenic lymphocytes contained little or no J chain or J chain-specific mRNA. In studies of human lymphoblastoid cell lines, however, McCune et al. (17) detected J chain in immunoglobulin-secreting, B cell, and pre-B cell lines. These variances prompted our studies of the morphological and biochemical correlation of J and μ chain expression as a function of differentiation along the human B cell axis (18–22).

Materials and Methods

Cells. Cells from peripheral blood (PB) and bone marrow (BM) of normal individuals, patients with acute lymphocytic leukemia (ALL), two patients with Sezary syndrome, and a patient with acute myelofibrosis, and two pre-B cell lines (Nos. 207 and 697) were examined. Lymphoid cells from PB were separated by centrifugation on Ficoll-Hypaque gradients. Immunologic characterization of the leukemic cells was performed by immunofluorescence to detect surface and cytoplasmic immunoglobulin, HLA-DR, B cell, common ALL, and a myelomonocytic antigen. T cells were enumerated by an E-rosette
assay. The methods used in determining the cell phenotypes are described elsewhere (23). Lymphocytes (10^6 cells/ml) from PB of normal individuals were also stimulated with pokeweed mitogen (PWM) (10 μl/ml) and the presence of J chain was determined on days 0, 2, 5, and 8 of culture.

In a previous study of J chain biosynthesis (24), we used human lymphoblastoid cell lines that secrete IgG, IgA, or IgM and contain intracellular J chain to establish optimal conditions for the detection of J chain in cell lysates by radioimmunoassay (RIA). To release intracellular contents, single-cell suspensions were washed three times in phosphate-buffered saline with 1% fetal bovine serum, adjusted to 10^7 cells/ml and disrupted by sonication at 0°C for 1 min at 20 kHz/sec at 90 W (Sonifer cell disruptor, model W140; Health Systems-Ultrasonics, Inc., Plainview, NY). The sonicates were centrifuged for 5 min at 12,800 g and assayed directly for J chain; another aliquot was reduced (with 10 mM dithiothreitol in 0.2 M Tris, pH 8.4, for 2 h at room temperature) and alkylated (with 21 mM iodoacetamide in 0.2 M Tris, pH 8.4, for 15 min) before the J chain RIA. Lysates of human T cell lines (MOLT-4, MOLT-3, and HSB), and an epithelial cell line from a human colonic carcinoma (HT-29) did not contain detectable J chain and were used as controls (24).

Antisera, RIA, and Immunohistochemistry. Rabbit antibodies specific for either human μ or J chains were prepared by immunization with IgM-λ protein purified from plasma of a patient with Waldenström’s macroglobulinemia, or with J chain isolated by a previously described procedure (25) from S-sulfonated polymeric IgA2-λ myeloma protein. The anti-IgM antibody was subsequently absorbed with IgG-λ myeloma protein and Bence-Jones λ chains covalently bound to cyanogen bromide-activated Sepharose 4B. The specificity of these reagents was tested by immunoelectrophoresis, immunofluorescence, and RIA (24, 26). The details of the RIA for J chain produced by human lymphoid cells were described previously (24). The sensitivity and range of J chain RIA was 0.2–25 ng/100 μl sample. This anti-J chain reagent was also used by McCune et al. (17) for the immunoprecipitation of [35S]methionine-labeled polypeptides produced in vitro and in vivo, and was found to selectively precipitate J chain.

For immunohistochemical studies, Fab' and F(ab')2 fragments of anti-μ and anti-J chain antibodies were labeled with horseradish peroxidase (HRP) (type VI; Sigma Chemical Co., Saint Louis, MO) according to the method of Nakane and Kawaoi (27). For detection of intracellular J and μ chains, cells were fixed with 3% glutaraldehyde in 0.2 M sodium cacodylate buffer, pH 7.35, for 30 min and treated with saponin (28). Cells were incubated subsequently with HRP-labeled Fab' or F(ab')2 fragments for 1 h at room temperature, washed with sodium cacodylate buffer, and reacted with 3,3'-diaminobenzidine tetrahydrochloride (DAB) (Sigma Chemical Co.) to demonstrate peroxidase activity (29). After the DAB reaction, the cell suspensions were fixed with 1% osmium tetroxide for 1 h, dehydrated with a graded ethanol and acetone series, and embedded in Spurr low viscosity resin (Ladd Research Industries, Inc., Burlington, VT). Thin sections were made with an ultramicrotome (Ultrotome III; LKB Instruments, Inc., Gaithersburg, MD) and examined, without counterstaining, in a Philips 300 electron microscope (TEM 300; Philips North America, Atlanta, GA). At least 600 cells from each preparation were examined.

In control experiments, cells from (a) PB and BM of normal individuals, (b) patients with ALL, Sézary syndrome, and acute myelofibrosis, and (c) leukemic cell lines were examined for the presence of endogenous peroxidase by the DAB reaction. Negative immunohistochemical controls included incubation of cells with DAB without hydrogen peroxide, incubation with heat-inactivated labeled antibodies, and incubation with HRP-labeled F(ab')2 fragments of the anti-α chain reagent.

Results

Presence of J Chain in Leukemic Cells Representative of Early B Lineage Stages of Differentiation. For the purpose of these studies, highly enriched preparations of cells representative of early stages in differentiation along the B cell pathway were required. Since it has not yet been possible to isolate precursor cells of B...
lineage from normal tissues, representatives of their malignant counterparts were selected for study. A very early stage in differentiation along the B cell axis would involve rearrangements of the immunoglobulin heavy chain genes, VH, DH, and JH that precede μ chain expression. Almost all leukemias of "null" (non-T, non-B) phenotype have features suggestive of an arrest at this stage in differentiation in that they have nonproductive rearrangements of heavy chain genes, with or without rearranged light chain genes (30, 31), and express B cell differentiation antigens on their surface (32, 33). For this study, four patients whose lymphocytes displayed null cell leukemic phenotype were selected. Pre-B leukemia cells represent the next stage in differentiation. These cells express heavy chains but usually not light chains (34, 35). We selected for study three representatives of this leukemic phenotype, all of which contained μ chains in their cytoplasm. None of these expressed light chains, but one population of leukemic cells bore trace amounts of μ chain on the surface, and may therefore represent a pre-B/B transitional phase. In addition, two well-defined pre-B leukemic cell lines (23, 36) were studied.

Intracellular J chain was detected in leukemic cells of both null and pre-B phenotypes (Table I). In HLA-DR+ null cells, J chain was detected in small and medium lymphocytes (7–9 μm); the majority of J chain-positive cells of BM origin were small lymphocytes, and those from blood samples were medium-sized lymphocytes (Fig. 1). J chain was associated with ribosomes of the leukemic cells from both tissues. However, among J chain-positive leukemic lymphocytes from BM, a small subpopulation (~7%) expressed J chain only on ribosomes bound to the membranes of the perinuclear cisternae (Fig. 1D). The presence of intracellular μ chain was detectable in <1% of cells in the HLA-DR+ null cell preparations.

In pre-B leukemic cells, J chain was detected on ribosomes distributed diffusely.

**Table I**

Presence of J Chain in Cells from Patients with ALL and in Pre-B Cell Lines

| Leukemic cells | Source | HLA-DR | Surface μ chain | Cytoplasmic μ chain | B cell antigen | Myelomonocytic antigen | Common ALL antigen | E-rosette formation | J chain* | Total* | Free |
|----------------|--------|--------|-----------------|--------------------|---------------|------------------------|------------------|-------------------|--------|--------|------|
| ALL-Null       | PB     | >90    | <1              | <1                 | <1            | >90                    | 5                | 88                | 20.0   | 6.7    |
| ALL-Null       | BM     | 98     | <1              | <1                 | <1            | 2                      | 98               | <1                | 24     | 12.5   |
| ALL-Null       | PB     | 51     | 5               | ND                 | 7             | 10                     | 40               | 21                | 94     | ND     |
| ALL-Null       | BM     | 100    | <1              | <1                 | ND            | <1                     | 60               | 8.8               | 1.5    |
| ALL-Pre-B      | BM     | 42     | <1              | ND                 | <1            | >90                    | <1               | 24                | 26.2   | 12.5   |
| ALL-Pre-B      | PB     | 54     | 6               | 97                 | 3             | 2                      | 75               | <1                | 2      | ND     |
| ALL-Pre-B      | NB     | >90    | ND              | 67                 | 12            | ND                     | <90              | ND                | ND     | 32     |
| ALL-Pre-B      | PB     | 88     | <1              | 82                 | ND            | ND                     | ND               | 80                | 12.5   | 7.8    |
| Pre-B          | Cell line 207 | 80     | <1              | 95                 | 5             | <1                     | 70               | <1                | 40     | 0.8    |
| Pre-B          | Cell line 697 | 47     | <1              | 87                 | 1             | <1                     | 70               | <1                | 40     | 6.5    |

* By electron microscopy.
† Reduced and alkylated sample analyzed by RIA.
‡ Not determined.
§ Average values of five examinations (cell line 207: range 36–44 percent; cell line 697: range 45–58 percent).
Figure 1. Subcellular distribution of J chain in HLA-DR-positive null leukemia cells. (A) J chain was detected on free ribosomes in medium-sized PBL. The cell in the upper left corner is J chain negative. × 11,000. (B) J chain-positive small lymphocyte from BM of the same patient. × 6,700. (C) Medium-sized lymphocyte from BM displays J chain on free ribosomes but the Golgi apparatus in shallow nuclear indentation is J chain negative. × 10,300. (D) Detail of two J chain-positive cells. The upper cell exhibits J chain only on ribosomes attached to the perinuclear cisterna (arrows); the lower cell contains J chain bound to free ribosomes. × 19,600.
FIGURE 2. Subcellular distribution of J chain in leukemic pre-B cells. (A) Medium-sized lymphocyte (L) from peripheral blood contains J chain on free ribosomes. The plasma cell (P) in the field contains J chain, which is located primarily within Golgi-derived vesicles (g). × 13,900. (B) Pre-B cell from leukemic cell line 207. Cytoplasm of the upper cell contains J chain-positive ribosomal clusters. × 16,300. (C) Pre-B/B leukemic cell is J chain positive on free ribosomes, whereas numerous short profiles of RER are J chain negative. × 8,500.
in the cytoplasm of small lymphocytes (7–8 μm) (Fig. 2A) or on ribosomal clusters and polysomes of large lymphoblasts (10–13 μm) (Fig. 2B). Mu chain was regularly found on free ribosomes and ribosomal clusters in both small and large pre-B cells. In leukemic pre-B cell lines, μ chain was often detected in tubules of rough endoplasmic reticulum (RER) of large dividing cells (Fig. 3, C and D). The Golgi apparatus of large cells also contained μ chain in vesicles of variable size (Fig. 3E).

**Subcellular Distribution of J and μ Chains in PWM-stimulated Lymphocytes from Normal Individuals.** Normal peripheral blood mononuclear cells were examined for the presence of J and μ chains, before and 2, 5, and 8 d after PWM stimulation (Table II). Increase in the numbers of cells expressing cytoplasmic J chain and μ chain was observed as a function of time after PWM stimulation. J chain-positive cells were sixfold more numerous than μ chain-positive cells at each time point examined. The increased proportions of J chain-positive cells was due to the fact that J chain was detectable in lymphocytes and lymphoblasts, whereas intracytoplasmic μ chain was primarily seen in immature and mature plasma cells. In addition, μ and J chains displayed different subcellular localization. In lymphocytic cells, J chain was detectable exclusively on free ribosomes and ribosomal clusters, and not in the Golgi apparatus or RER, when the latter organelle was present. In plasma cells, J chain was seen in RER or the Golgi apparatus, and not on free cytoplasmic ribosomes. The simultaneous presence of J chain in RER and in the Golgi apparatus was seen only occasionally (Fig. 4). In contrast, μ chain was regularly found in both RER and the Golgi apparatus of plasma cells on days 5 and 8 (Fig. 5).

Only 0.3 and 3.7% of the mononuclear cells isolated from the PB of two patients with Sezary syndrome contained J chain; 2.5% of the BM cells from the latter patient contained J chain. BM from a patient with acute myelofibrosis contained 0.4% J chain-positive cells.

**Biosynthesis of J Chain.** The levels of J chain present in lysates of null and pre-B ALL cells, two pre-B cell lines, and PWM-stimulated PB lymphocytes (PBL) are presented in Tables I and II. J chain was detected by RIA in all of these lysates, but not in lysates of non-B cell lines examined previously (24). The ALL null and pre-B cells differed from PWM-stimulated lymphocytes from PB of normal individuals with respect to the amount and molecular form of intracellular J chain. Although 21–88% of null and pre-B cells contained J chain identifiable by immunoelectron microscopy, only 8.8–34.5 ng J chain per 10^7 cells was measured by RIA. By comparison, 6% of unstimulated lymphocytes from PB displayed J chain and this sample contained 6.6 ng/10^7 cells. After PWM stimulation, 57% of the cells were positive for J chain by immunoelectron microscopy on day 8, and the amount of intracellular J chain increased to 128 ng/10^7 cells. These values indicate that in null, pre-B, and B cells, the J chain levels are considerably lower than those present in PWM-stimulated PBL.

We have shown previously that reduction and alkylation results in: (a) markedly increased intensity of intracellular immunofluorescence of cells stained with fluorochrome-labeled anti-J chain (11); (b) exposure of originally inaccessible antigenic determinants of J chain in polymeric myeloma IgA, secretory IgA, and IgM (26); and (c) detectability by RIA of intracellular J chain in lysates of human
FIGURE 3. Subcellular distribution of μ chain in leukemic pre-B cells. (A) A single μ chain-positive pre-B cell from PB exhibits the positivity on free ribosomes. × 8,500. (B) A large pre-B cell from BM contains ribosomal clusters that are μ chain positive. × 10,200. (C) A large pre-B cell from leukemic cell line 697 during mitotic division. The positivity for μ chain is expressed within long tubules of RER. (c-chromatin material). × 11,200. (D) Detail of a pre-B cell from leukemic cell line 207. The two short tubules of RER containing μ chain are in close apposition to the cell surface, one of them merging with cell membrane (arrow). × 26,500. (E) The vesicles in the Golgi region of a pre-B leukemic cell line 207 contain μ chain. × 17,100.
Expression of J Chain and µ Chain by Normal PBL Before and After Stimulation with PWM

| Culture interval in days | Intracellular J chain (EM)* | Intracellular µ chain (EM)* | Amounts of J chain (ng/10^7 cells) |
|-------------------------|-----------------------------|-----------------------------|-----------------------------------|
|                         | Percent positive cells | Positive cell type* | Percent positive cells | Positive cell type* | Intracellular | Secreted |
|                         |                       |                             |                       |                             | Total* | Free | Total* | Free |
| 0                       | 6                      | L (100)                     | 1                     | L (100)                     | 6.6   | 1.6  | ND    | ND   |
| 2                       | 34                     | L, LB (100)                 | 5                     | L, LB (100)                 | ND    | ND   | 65.0  | 0    |
| 5                       | 49                     | PC (5)                      | 9                     | PC (88)                     | 35.0  | 15.0 | 65.0  | 0    |
| 8                       | 57                     | LB (95)                     | 11                    | LB (6)                      | 128.0 | 73.0 | 280.5 | 0    |

* Electron microscopy.

**Total J chain content was measured after the reduction and alkylation of cell lysates or culture supernatants; free J chain was measured in unreduced samples of these fluids.

* L, lymphocytes; LB, lymphoblasts; PC, immature and mature plasma cells. Numbers in parentheses indicate the relative distribution of the cell types that express intracellular J or µ chains.

Discussion

Results of this study indicate that J chain is detectable by immunoelectron microscopy and RIA in human lymphoid cells that represent the earliest recognizable developmental stages along the B cell axis. J chain was found in HLA-DR+ null leukemia cells, pre-B leukemia cells, normal B cells, and immature as well as mature plasma cells. These observations confirm the earlier study of McCune et al. (17) and indicate that J chain is expressed in precursors of human B cells. The failure to detect J chain in such cells by immunofluorescence (4), as opposed to in vivo pulse labeling, in vitro translation of total cellular RNA, immunoelectron microscopy, and RIA, may reflect differences in sensitivities of these techniques. Furthermore, antigenic determinants of intracellular J chain may be masked by association with immunoglobulins or with unidentified cell components. Detection of increased numbers of J chain-positive cells and the increased intensity of staining after incubation with acid-urea (3, 37), reduction
Figure 4. Subcellular distribution of J chain in PWM-stimulated PBL. (A) A small lymphocyte on the 2nd d of culture is positive for J chain on free ribosomes. The Golgi apparatus near the nuclear indentation is negative. × 13,700. (B) A lymphoblast from the 2nd d of culture contains J chain on non-membrane-associated ribosomes and ribosomal clusters distributed throughout the cytoplasm. × 8,500. (C) Lymphoblastoid cell on day 5 in culture contains J chain on free ribosomes and ribosomal clusters, whereas the Golgi apparatus and RER are negative. × 8,400. (D) A mature plasma cell on day 8 of culture. J chain is localized in a well-developed RER system. × 13,100. (E) Detail of the Golgi apparatus of a mature plasma cell on day 8 in culture with J chain-positive tubules and vesicles. × 24,000.
FIGURE 5. Subcellular distribution of \( \mu \) chain in PWM-stimulated PBL. (A) An immature plasma cell from 5-d culture. Mu chain is present in long narrow tubules of the RER. \( \times 14,600 \). (B) A mature plasma cell on day 8 culture contains \( \mu \) chain both in widened RER tubules and in the Golgi apparatus (g). \( \times 10,800 \). (C) On day 8 in culture, the \( \mu \) chain-containing RER channels are wide and distended up to the cisternae in many of the plasma cells. \( \times 24,000 \). (D) From day 5 in culture, many of the plasma cells contain \( \mu \) chain in RER tubules and in the Golgi apparatus. \( \times 15,100 \).
and alkylation (11), or mild pretreatment of tissue sections with trypsin (38)
support this contention. Such treatment is particularly effective in exposing J
chain determinants in B cell blasts. For example, Brandtzaeg (37) observed that
exposure of unstimulated human PBL to acid-urea reduced the numbers of J
chain-negative IgA blasts from 21–50% to 3–13%. In the null leukemia cells
that we examined, J chain was apparently bound to nonimmunoglobulin cellular
components since the immunoglobulin gene rearrangements in such cells are
nonproductive (30). With respect to intracellular distribution, J chain was de-
tected by immunoelectron microscopy in null and pre-B cells on free ribosomes
and ribosomal clusters, and on perinuclear cisterna-bound ribosomes, but not in
the Golgi apparatus. In PWM-stimulated PBL, J chain was found on non-
membrane-associated ribosomes in lymphoid cells, whereas in plasma cells it was
detected within RER and rarely in the Golgi region. Our results are in agreement
with those of Nagura et al. (39) who described a similar distribution of J chain
in unstimulated lymphocytes and in plasma cells from rectal biopsies. In contrast,
μ chain was detected in association with ribosomes within PWM-induced lympho-
blasts, and in RER and the Golgi apparatus of plasma cells. Dissimilarity in the
subcellular distributions of μ and J chains may be related to differences in
intracellular kinetics and mechanisms of secretion. J chain has never been
detected in supernatants of cultured lymphoid cells unless attached to polymeric
immunoglobulins (13, 24) and is degraded in cells that produce monomeric
immunoglobulins (40).

The presence of mRNA as well as translated J chain in cells that do not produce
immunoglobulins (15, 17, this paper) indicates that expression of these proteins
can be independently regulated during differentiation of cells along the human
B cell pathway. Recently, Yagi et al. (41) have shown that a single J chain gene
per haploid genome is located on mouse chromosome 5 and is not linked to
immunoglobulin heavy and light chain structural genes. A functional advantage
for the expression of J chain, before expression of μ chain in B cell precursors,
is not immediately evident, but cannot be related to immunoglobulin polymeri-
zation.

These results are at variance with the proposal that J chain synthesis is initiated
during the differentiation of B cells into immunoglobulin-secreting cells as a
consequence of mitogenic or antigenic stimulation (3, 16, 42). Because the J
chain-positive null and pre-B leukemia cells lack cell surface immunoglobulins,
and probably receptors for mitogens and T cell factors as well, J chain expression
would appear to precede the antigen-induced series of events that leads to
maturation into immunoglobulin-producing cells. Nevertheless, a remarkable
increase in synthesis of J chain in mitogen-stimulated cells parallels the production
of immunoglobulins, regardless of their class (14, 24). J chain synthesis is
preserved in both normal and malignant cells that produce monomeric immu-
oglobulins, or only heavy or light chains (4, 9–15, 24, 42). Once initiated, J
chain expression in cells of B lineage apparently persists even in mature plasma
cells secreting immunoglobulin molecules devoid of J chain. For example, PWM-
induced, IgG-producing cells expressed J chain as did plasma cells that produced
IgM or IgA. While J chain could participate, in collaboration with disulfide-
interchange enzymes (43, 44), in the formation of intra- or inter-chain disulfide
bonds of heavy and light chains, any additional role(s) remains to be determined.

The expression of J chain in lymphoid cells that do not produce polymeric immunoglobulins could be attributed to malignant transformation. However, malignant cells of myeloid or T cell lineages did not contain detectable J chain. Furthermore, the presence of intracytoplasmic J chain in resting B lymphocytes and in nonmalignant cells that produce monomeric immunoglobulins is more consistent with the view that early J chain synthesis is the rule for B cell differentiation in humans.

Summary

J chain expression was examined as a function of the stage in differentiation along the B cell axis in humans. Intracellular distribution of J and \( \mu \) chains in leukemic HLA-DR\(^{+}\) null and pre-B cells, and in normal B cells stimulated with pokeweed mitogen (PWM) was determined by immuno-electron microscopy and radioimmunoassay (RIA). J chain was detected in leukemic null and pre-B cells on free and membrane-bound ribosomes in the cytoplasm, or on perinuclear cisternae. Mu chain was found on free ribosomes and ribosomal clusters in leukemic pre-B cells but was absent in the leukemic null cells. In pre-B cell lines, \( \mu \) chain was seen within rough endoplasmic reticulum (RER) and the Golgi apparatus whereas J chain was not detected in these organelles. However, both \( \mu \) and J chain were detected in RER and the Golgi apparatus of immature and mature plasma cells induced by PWM stimulation of normal peripheral blood lymphocytes. Low levels of J chain were also detected by RIA in lysates of leukemic null and pre-B cells. Most of the intracellular J chain became detectable after reduction and alkylation of cell lysates, and free J chain was not found in the culture supernatants. The amount of intracellular and secreted immunoglobulin-bound J chain increased dramatically after PWM stimulation of peripheral blood lymphocytes. The majority of J chain-positive cells seen over an 8 d culture interval were lymphocytes and lymphoblasts, while \( \mu \) chain was found primarily in plasma cells. These results suggest that J chain expression precedes \( \mu \) chain synthesis during B cell differentiation and that a combination of the two chains for secretion is not initiated until the onset of plasma cells maturation.

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