PARALLEL SYNTHESIS OF IMMUNOGLOBULINS AND J CHAIN IN POKEWEED MITOGEN-STIMULATED NORMAL CELLS AND IN LYMPHOBLASTOID CELL LINES

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In sera and in secretions, J chains are found linked to molecules of polymeric IgA and IgM by disulfide bonds. Their function remains undetermined; the initial concept that they were essential to the process of polymerization has been questioned. It is of special interest that J-chain synthesis is not restricted to cells producing polymeric immunoglobulins of the IgA and IgM classes; it has been detected in murine plasma cells that produce IgG as well as in those that synthesize only L chains (1-4). Within such cells, J chain was thought to be either linked to a small portion of intracellular immunoglobulin or in an unbound form, but it was not secreted except in association with polymeric immunoglobulins. In sections of human lymphoid tissues, J chain was found in the majority of cells that contained immunoglobulins (5).

The present studies were undertaken to explore the relation of J chain and immunoglobulin synthesis during the process of differentiation of normal B cells to plasma cells as a result of pokeweed mitogen stimulation. Studies were also performed on various B-cell lymphoid lines wherein immunoglobulin-synthesizing plasma cells represent a minor population related to the cell growth cycle (6).

Materials and Methods

Preparation and Incubation of Cells. Human mononuclear cells were isolated from heparinized venous blood by Ficoll-Hypaque gradients. Cell surface immunoglobulins were detected by fluorochrome-labeled F(ab')2 fragments of rabbit antibodies using viable lymphocytes (7). The cell mixture had been previously incubated with latex as an aid in the identification of monocytes (7). For the detection of intracellular immunoglobulins and J chain, 0.02 ml of 5 × 10⁶ cells/ml suspended in phosphate-buffered saline (PBS) containing 1% bovine serum albumin (BSA) and 0.1% sodium azide were subjected to cytostaining. The cells were fixed for 20 min at -20°C in a mixture of 100 ml of 95% ethanol and 5 ml of glacial acetic acid, washed three times in cold PBS, and left overnight in the same solution. The cells were stained for 1 h with fluorochrome-labeled reagents and washed three times with PBS. After overnight washing with PBS, the slides were mounted in buffered elvanol solution.

For tissue cultures 1 × 10⁷ peripheral blood lymphocytes were suspended in 10 ml of tissue culture medium RPMI 1640 supplemented with 10% fetal calf serum, penicillin, and streptomycin (Associated Biomedic Systems, Inc., Buffalo, N. Y.) in Falcon tissue culture flasks (Falcon Plastics, Oxnard, Calif) and incubated at 37°C in 5% CO₂. To 10 ml of tissue culture, 0.1 ml of

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pokeweed mitogen (PWM) (Grand Island Biological Company, Grand Island, N. Y.) or 0.2 ml of PHA (Wellcome Reagents, Ltd., Beckenham, England) was added.

Cells from established cultures of lymphoblastoid cell lines were processed for surface or intracellular staining as described for peripheral blood lymphocytes.

**Reagents** Antisera to human J chain were produced in rabbits. J chain was isolated from an IgA2\(\lambda\) polymeric myeloma protein (8) and coupled to BSA as described by Brandtzaeg (5). Rabbits were immunized three times, at 1-mo intervals, with the antigen in complete Freund's adjuvant. The antiserum was absorbed on an immunosorbent column consisting of BSA, attached to CNBr-activated Sepharose 4B. The methods used for the preparation of \(\gamma\)-globulin fractions and F(ab')\(_2\) fragments of rabbit antisera to human immunoglobulins, their conjugation with fluorochromes, and absorption with insolubilized antigens have been described (7) Conjugates of undigested antibody molecules were used for intracellular staining, and F(ab')\(_2\) antibody fragments were used for surface fluorescence. An additional absorption with mouse liver powder was used to further decrease nonspecific intracellular staining. The specificity of the F(ab')\(_2\) reagents was verified using cell surface fluorescence of monoclonal lymphoid cells, whereas that of the whole antibody reagents was defined on tissue sections. Furthermore, the specificity of staining was tested after absorption of the antiserum with insolubilized antigens and by blocking with unconjugated antisera; in particular, J-chain staining was inhibited by absorption with S-sulfonated J chain and with polymeric IgA2\(\lambda\) or IgA2\(\kappa\) proteins, but not with \(\lambda\)-chains or pooled IgG To determine the distribution of J chain in cells that contained different individual immunoglobulin classes, double tetramethylrhodamine isothiocyanate (TRITC)-labeled anti-J-chain reagents. Polyvalent rabbit antiserum to IgG + IgM + IgA with antibodies to both H and L chains was obtained from a commercial source (Behring Diagnostics, Woodbury, N Y.).

**Results**

*Immunoglobulin and J Chain in Unstimulated and Mitogen-Stimulated Peripheral Blood Leukocytes.* Intracellular immunoglobulin was detected in an average of 0.4% of unstimulated lymphocytes; of these, immunoglobulin class distribution was variable but always included cells positive of IgM, IgA, and IgG. In double label experiments using either a polyvalent anti-immunoglobulin or class-specific reagents, all the cells containing immunoglobulins also stained positively for J chain irrespective of the class of intracellular immunoglobulin present. Surface staining of peripheral blood lymphocytes (PBL) was not observed with the TRITC-labeled F(ab')\(_2\) fragment of anti-J chain, except in a single instance, when weak staining was seen in a small percentage of lymphocytes. This staining was not detectable when overnight cultivation preceded the staining with anti-J-chain antiserum.

After PWM stimulation of PBL from three subjects, the number of cells that contained immunoglobulin and J chain exhibited a progressive and parallel increase from day 3 until day 6, when a plateau was reached (Fig. 1). In double label experiments, no cells were observed in which staining for J chain was found in the absence of immunoglobulins. However, after 7-10 days of culture, J chain was undetectable or weakly positive in a portion of immunoglobulin-positive cells regardless of their immunoglobulin class (Fig. 1). The proportion of cells that contained IgM, IgG, and IgA immunoglobulin remained virtually unchanged, irrespective of the day of examination (Table I). To exclude the possibility that the J chain found in IgG-producing cells reflected a residuum of prior synthesis of IgM or IgA immunoglobulins, the cells were stained successively at different times with FITC-labeled anti-\(\alpha\) and anti-\(\mu\)-chain, and TRITC-labeled anti-J chain. The number of cells that contained J chain consistently
Fig. 1. Percentage of cells containing immunoglobulin and J chain in PWM-stimulated PBL of two healthy individuals. The immunoglobulin-containing cells (●—●) were disclosed with the use of FITC-labeled polyvalent antiserum to H and L chains, and J chain (○—○) with TRITC-labeled anti-J chain. Exact correspondence in percentages was observed through the first 3 days.

TABLE I

| Days | Ig-positive* cells | Distribution of Ig classes in these cells$ | IgM | IgA | IgG | IgM + IgA$
|------|-------------------|--------------------------------------------|-----|-----|-----|----------------|
| 0    | 0.4              | %                                          | %   | %   | %   | %              |
| 1    | 0.8              | 12  | 45  | 40  | 65  |
| 2    | 1.6              | 64  | 45  | 57  |
| 3    | 3.3              | 41  | 56  |
| 4    | 6.8              | 48  | 53  |
| 5    | 12.3             | 58  | 56  | 48  |
| 7    | 15.9             | 47  | 38  | 64  |
| 8    | 18.2             | 38  | 41  | 56  |
| 10   | 18.2             | 38  | 56  | 64  |

* Percentage of PBL containing intracellular immunoglobulin was disclosed with an FITC-labeled polyvalent antiserum.
$ The distribution (in percent) of various immunoglobulin classes in these cells was determined by the staining with H-chain-specific fluorochrome-labeled antisera.
$ Determined by consecutive staining with $\mu$- and $\alpha$-chain-specific antisera.

exceeded the number of IgM- and IgA-producing cells in a proportion that corresponded to the number of IgG cells. This indicates that cells producing only IgG also synthesize J chain. In contrast to this intracellular J chain in the final preparations, no J chain was detectable on the cell surface. Less than 0.5% of the cells contained immunoglobulins and J chain in the 7-day cultures of PBL stimulated by phytohemagglutinin (PHA). In one patient with common variable hypogammaglobulinemia and one with isolated IgA deficiency, intracellular immunoglobulin and J chain were present together in 1.6 and 5.7% of the cells, following PWM stimulation, respectively. No immunoglobulin or J-chain pro-
TABLE II
Surface-Associated and Intracellular Immunoglobulins and J Chain in Lymphoblastoid Cell Lines

| Cell line | Surface Immunoglobulin | Surface J chain | Intracellular Immunoglobulin | Intracellular J chain |
|-----------|------------------------|-----------------|-----------------------------|------------------------|
| HLHL-1    | IgM 18%                | - *             | IgM 14%                     | 14%                    |
|           | IgD 4%                 |                 | (3+)                        | (4+)                   |
| Daudi     | IgM 90%                | -               | IgM tr§                     | tr                     |
|           | (4+)                   |                 |                             |                        |
| Wil-2     | IgM 24%                | -               | IgM 5%                      | 5%                     |
|           | (tr)                   |                 | (2+)                        | (3+)                   |
| 32a1      | IgA 86%                | -               | IgA 16%                     | 16%                    |
|           | (1-3+)                 |                 | (2+)                        | (2+)                   |
| 32a7      | IgA 46%                | -               | IgA 13%                     | 13%                    |
|           | (1-2+)                 |                 | (3+)                        | (3+)                   |
| 8866      | IgG 16%                | -               | IgG 17%                     | 14%                    |
|           | (tr)                   |                 | (2+)                        | (3+)                   |
| HSB       | IgG 18%                | (tr)            | IgG 7%                      | 7%                     |
|           | IgM 5%                 |                 | (1-2+)                      | (1+)                   |
| SB(T)     | -                      |                 | -                           | -                      |

* Not detectable
† Relative intensity of fluorescence on 1+ to 4+ scale
§ Traces

Reduction was detected in three other patients with common variable immune deficiency or one patient with Bruton type hypogammaglobulinemia.

Immunoglobulin and J-Chain Synthesis in Established Lymphoblastoid Cell Lines. Despite a variation in the percentage of immunoglobulin-positive cells among B-type lymphoid lines, the number of positive cells was parallel to the number of cells that contained J chain, and in double label experiments an exact correspondence between J chain and immunoglobulin was observed (Table II). J chain, however, was not demonstrable on the surface of any lymphoid line, including those expressing large amounts of surface immunoglobulin. In particular, the Daudi cell line that had abundant surface IgM, but no intracytoplasmic IgM, lacked detectable J chain either on the cell surface or intracellularly.

Discussion

The results of this study indicate a remarkable parallelism between the development of immunoglobulin synthesis and of J-chain synthesis during the process of differentiation of B cells after mitogen stimulation. The time of appearance of mature plasma cells containing these two proteins coincided exactly, and the two appeared together in the same cells. Initially, there was complete concordance between the two, and every cell containing immunoglobulin in the cytoplasm also contained J chain. At later times after mitogen stimulation, a few immunoglobulin-positive cells appeared to lack J chain although the reverse was never found. Before stimulation the number of PBL-
containing intracellular immunoglobulins, the distribution of individual classes, and the presence of J chain in these cells correspond closely to results reported previously (9, 10). Similarly, J-chain determinants were not disclosed on the surface of unstimulated, PWM-stimulated B cells, or long-term B-cell lymphoid lines.

In the lymphoid cell lines studied, the same parallelism between J chain and immunoglobulin was evident. Here, too, evidence is available that differentiation occurs during the cell cycle. Recent work from our laboratory indicates that these cultures, when unsynchronized, represent a mixture of cells some of which carry surface immunoglobulins and others, which resemble typical plasma cells, lack surface immunoglobulin but contain large amounts of cytoplasmic immunoglobulin. Marked changes in the ratio of these cell types occur when the cultures are synchronized. The J chain appears only with the development of the plasma cells and is not detectable in lines, such as Daudi, with abundant surface IgM. Mitogen stimulation with PHA of T cells to blast cells did not induce the synthesis of immunoglobulins or J chain, which were also absent from the T-cell line (SB).

It was apparent, in this study, that plasma cells synthesizing either IgM, IgG, or IgA were also involved in J-chain synthesis. The reason for J-chain synthesis in parallel to immunoglobulin synthesis, even in cells that produce monomeric immunoglobulins, is not clear. Because of the covalent association with polymeric serum and secretory immunoglobulins, it was assumed originally that J chain is essential to the process of polymerization (11). Although this hypothesis has not been excluded, the existence of serum polymeric immunoglobulins that lack J chain (12), J-chain synthesis in cells that produce nonpolymeric IgG and IgD or only L chains (1-3, 5, 9, 10), and in vitro combination of polymeric immunoglobulins in the absence of J chain (13) suggest that J chain may have other functions in addition to the mediation of polymerization. The current studies on the very close relationship to immunoglobulin synthesis during the process of B-cell differentiation strongly suggest an important and as yet unidentified role for J chain in the process of immunoglobulin synthesis. One possibility is that, during active synthesis of intracellular immunoglobulins, J chain may mediate the formation of not only the disulfide bonds between monomeric subunits but also the assembly of the Ig chains. It is of interest that J chain has been detected bound to α- and μ-chain disease proteins which are not polymeric (14). Further studies, especially those directed to possible associations with various intracellular immunoglobulins at different stages in the formation of the final molecule, may provide an answer.

Summary

The synthesis of intracellular J chains was found to be closely associated with that of intracellular immunoglobulin, regardless of its class, during the process of B-cell differentiation. This parallelism between the synthesis of J chain and immunoglobulin was particularly evident in their coincident appearance in serial observations of pokeweed mitogen (PWM)-stimulated lymphocytes. The

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1 Fu, S. M., J. Hurley, and H. G. Kunkel. Relationship between surface and intracellular immunoglobulins in B cell lymphoid lines. Manuscript in preparation.
intensity of J-chain staining by fluorescent reagents in the stimulated cells synthesizing IgG was similar to that found in cells synthesizing IgA or IgM. Evidence was obtained that the presence of J chain in the IgG-producing cells did not reflect antecedent synthesis of IgA or IgM. T cells stimulated by phytohemagglutinin and PWM failed to show J-chain synthesis. Observations on lymphoid cell lines showed a similar parallelism between intracellular Ig and J-chain synthesis; no relation to surface Ig was found.

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