The Journal of Immunology

RESEARCH ARTICLE | JANUARY 15 1991

Human B cells secrete predominantly lambda L chains in the absence of H chain expression. FREE

D M Ambrosino; ... et. al

J Immunol (1991) 146 (2): 599–602.

https://doi.org/10.4049/jimmunol.146.2.599

0022-1767/91/1462-0599$02.00/0
THE JOURNAL OF IMMUNOLOGY

Vol. 146, 599–602, No. 2, January 15, 1991
Printed in U.S.A.

HUMAN B CELLS SECRETE PREDOMINANTLY λ L CHAINS IN THE ABSENCE OF H CHAIN EXPRESSION[1]

DONNA M. AMBROSINO,[2]* M. V. KANCHANA,* NANCY R. DELANEY,* AND ROBERT W. FINBERG*

*From the *Laboratory of Infectious Diseases, Dana-Farber Cancer Institute, Boston, MA 02115*

Ig H and L chains are independently assembled in B cells and then secreted together as a functional protein. H chains cannot be secreted without assembly to L chains; however, L chains can be secreted in the absence of H chains by both mice and human cells. To examine the influence of H chain expression on human L chain isotype selection (κ or λ), we compared the κ/λ ratio of L chains unassociated with H chains (free L chains) to the κ/λ ratio of L chains associated with H chains. Culture supernatants of human splenocytes were assayed for κ and λ L chains. Free L chains were the predominant form of L chains detected in unstimulated cultures, accounting for 68 to 70% of the total. This was in contrast to the minor proportion that free L chains represented (<20%) in cultures stimulated with PWM or LPS ($p < 0.01$). Furthermore, the κ/λ ratio of light chains detected in unstimulated cultures was 0.5 as compared to 1.3 for PWM stimulated cultures ($p = 0.0001$). To demonstrate that the decreased κ/λ ratio of L chains in the supernatants of cultures of unstimulated B cells was due to free L chains, we measured the κ/λ ratio of IgG and IgM-associated L chains. In both the stimulated and unstimulated cultures, the κ/λ ratio of L chains associated with H chains was greater than the ratio determined for free L chains. Free L chains were shown to be predominantly λ as compared to the predominantly κ phenotype of L chains associated with H chains. Thus absence of H chain expression affects selection of L chain isotypes secreted by human B cells.

Ig H and L chains are independently assembled in B cells and then secreted together as a functional protein. Evidence now suggests that H and L chain gene rearrangements occur in an orderly fashion. H chain Ig genes rearrange first, followed by κ L chain gene rearrangement (1). If no productive κ gene rearrangement occurs, then λ L chain genes rearrange (2). After the rearrangements have occurred in pre-B cells, further B cell maturation is accompanied by H chain and L chain production. Of note, H chains cannot be secreted without assembly to L chains although L chains can be secreted in the absence of H chains in both human and murine B cells (3, 4). In this report we examined the production of L chains unassociated with H chains (i.e., free L chains) and noted a significant difference in the κ/λ ratio of free L chains compared to those associated with H chains.

MATERIALS AND METHODS

Cell separations. Human spleens were obtained from patients undergoing splenectomy for Hodgkin's disease, hereditary spherocytosis, or idiopathic thrombocytopenic purpura. Spleens were minced through wire mesh to create single-cell suspensions and then placed over Ficoll-Hypaque gradients (LSM; Organon Teknika Corporation, Durham, NC) (5). T cell-enriched populations were obtained by 12-h rosette formation with aminoethylisouronium bromide-treated sheep E followed by Ficoll-Hypaque gradients. The mononuclear layer (B cell enriched) and the pellet (T cell enriched) were harvested and the erythrocytes lysed with cold tris ammonium chloride buffer. Further purification of B cells were obtained using Percoll density discontinuous gradients. The phenotypes and functional characteristics of cells prepared in this fashion have been previously described (6). Each B cell fraction contained predominantly B cells (50 to 90% $CD20^+$ cells, and <2% $CD3^+$ cells). T cell-enriched populations contained predominantly T cells (>80% $CD3^+$ cells). As no differences were observed for B cells obtained at different densities, the data were pooled.

Cell cultures. Purified B cells at 1×10^6 cells/well were cultured with an equal number of enriched T cells in 2 ml of RPMI 1640 media (Mediatech, Washington, DC) supplemented with selected 20% heat-inactivated FCS (GIBCO, Grand Island, NY), 100 μg/ml penicillin, 100 μg/ml streptomycin in 24-well plates (Costar, Cambridge, MA). Cultures were stimulated with PWM (GIBCO) at a 1/200 final dilution or LPS (*Escherichia coli* 0111:B4, List Biologicals, Campbell, CA) at 10 μg/ml. B cells harvested from identical densities were compared. After 7 days of culture at 37°C and 5% CO_2, the supernatants were harvested and centrifuged to remove cells.

Antibody assays. The cell culture supernatants from four wells were pooled and concentrated 20-fold by SAS[3] precipitation. Overnight precipitation in 50% SAS was followed by two washes in 50% SAS and centrifugation at 10,000 × *g* for 20 min. The pellets were resuspended in PBS and dialyzed against PBS extensively. The concentrated supernatants were then assayed for total IgG, IgM, IgA, and total κ and λ Ig (using anti-κ or anti-λ capture antibodies) as previously described (7). To measure IgG-associated κ or λ L chains, microtiter plates were coated with anti-IgG capture antibody and developed with anti-κ or anti-λ alkaline phosphate conjugates (Tago, Burlingame, CA). An analogous system was used to measure IgM associated κ and λ L chains. The κ and λ myeloma proteins (WHO/IWIG subcommittee reference preparations) were used to standardize the L chain assays and establish specificity.

RESULTS

κ and λ Ig concentrations. We first determined the concentration of total κ and λ L chains in the supernatants from cultures of splenocytes of six spleens. The ELISAs for κ and λ L chains were shown to detect L chains associated with H chains as well as unassociated (free) L chains. Sensitivity and specificity was demon-

Received for publication June 1, 1990.
Accepted for publication October 15, 1990.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
[1] This work was supported by the National Institutes of Health Grant AI24659 and the World Health Organization Grant V23/181/15.
[2] Address correspondence and reprint requests to Dr. Donna Ambrosino, Dana-Farber Cancer Institute, 44 Binney St., Boston, MA 02115.

[3] Abbreviation used in this paper: SAS, saturated ammonium sulfate; FCS, fetal calfgerum; LPS, lipopolysaccharide; PWM, pokeweed mitogen.

strated using κ and λ myeloma proteins (WHO/IWIG subcommittee reference preparations) and purified Bence-Jones proteins (kindly supplied by Dr. Peter Schur, Brigham and Women's Hospital; Boston, MA). The assays were standardized and the specificity determined using these reagents (Fig. 1).

In unstimulated cultures we were surprised to find that λ L chains were predominant over κ L chains (Fig. 2). The geometric mean κ/λ ratio of 15 unstimulated cultures was 0.5 which was significantly lower than 1.3 for 15 cultures stimulated with PWM ($p = 0.0001$ by t-test). Eleven of these 15 cultures were also stimulated with lipopolysaccharide (LPS) and the κ/λ of the unstimulated cultures were again significantly lower than the lectin stimulated cultures ($p = 0.001$ by t-test) (Fig. 2).

IgG and IgM concentrations. We then determined the concentration of IgG and IgM in the supernatants of the cultures stimulated with PWM or LPS (Table I). As has been shown by others, PWM and LPS induced predominantly IgG and IgM responses, respectively (8). The IgG/IgM ratio of 1.4 for PWM-stimulated cultures was significantly greater than that of 0.2 for LPS ($p < 0.01$). Ig secretion induced by PWM and LPS were T cell dependent, consistent with previous reports (8). Cultures of purified B cells (with less than 2% T cells by flow cytometry) did not respond to LPS or PWM by enhanced production of IgG or IgM.

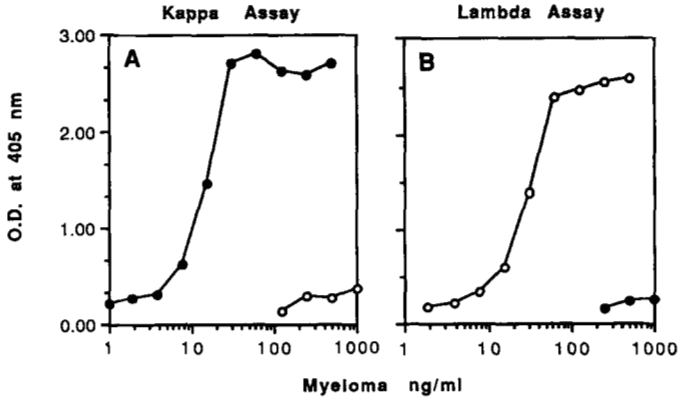

Figure 1. The κ and λ L chain ELISA. *A*, The OD reading in the κ ELISA for an IgG κ myeloma protein (*closed circles*) is compared to IgG λ myeloma (*open circles*). *B*, The OD readings in the λ L chain ELISA for an IgG λ myeloma protein (*open circles*) is compared to an IgG κ myeloma (*closed circles*).

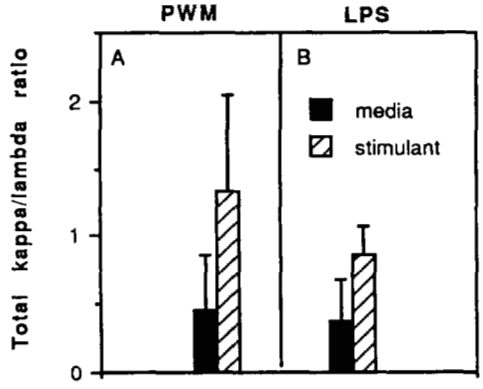

Figure 2. The κ/λ L chain ratio in supernatants of cultures of human B cell and T cells. *A*, The geometric mean κ/λ ratio (±SD) of 15 cultures stimulated with PWM as compared to 15 cultures without PWM. *B*, The geometric mean κ/λ ratio (±SD) of 11 cultures stimulated with LPS as compared to 11 cultures without LPS.

TABLE I

In vitro concentrations of Ig in cultures of splenocytes with PWM and LPS[a]

		Geometric Mean Antibody (μg/ml)				
	N	IgG	IgM	IgG/IgM	κ	λ
PWM stimulation						
Medium	15	0.4	1.0	0.4	1.9	4.2
PWM	15	207	144	1.4[b]	319	239
LPS stimulation						
Medium	11	0.4	1.0	0.4	1.8	4.8
LPS	11	2.2	11.9	0.2[b]	9.4	11.0

[a] Equal numbers of purified B cells and enriched T cells (1×10^6 cells) were cultured in 2 ml of medium for 7 days with PWM or LPS and the 20-fold concentrated supernatants were assayed.

[b] P value < 0.01 by Mann-Whitney rank sum test as compared to each other.

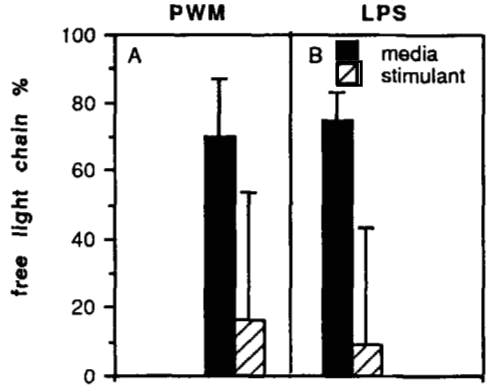

Figure 3. The percentage of total L chains that are free L chains in supernatants of cultures of human splenocytes. *A*, The mean total L chain percentage that are free (±SD) in supernatants of 15 cultures stimulated with PWM is compared to 15 unstimulated cultures. *B*, The mean total L chain percentage that are free (±SD) in supernatants of 11 cultures stimulated with LPS is compared to 11 unstimulated cultures.

κ/λ ratio of free L chains. The amount of κ and λ L chains in the supernatants of unstimulated cultures exceeded the quantity of IgG and IgM measured in these supernatants. This suggested substantial amounts of free L chains (L chains unassociated with H chains) were secreted. We therefore calculated the percentage of total L chains that were "free" in these cultures by subtracting the amount of total Ig measured (IgG and IgM) from the amount of total L chains measured (κ and λ).

We determined that L chains detected in the supernatant of splenocytes existed predominantly as free L chains, accounting for 68 to 70% of the total (Fig. 3). However, after lectin stimulation the percentage of L chains that were free fell to <20%. The decrease in free L chain percentage for PWM- and LPS-stimulated cultures was significant when compared to unstimulated cultures ($p < 0.01$). We therefore hypothesized that the significant decrease in the κ/λ ratio of total L chains in unstimulated cultures was the result of an abundance of free L chains in these cultures. Furthermore, we proposed that the free L chains must be predominantly λ. This λ predominance of free L chains observed in unstimulated cultures was not dependent on T cells as purified B cells cultured without T cells had a similar κ/λ ratio of 0.3.

Of note, the small amount of IgA-associated L chains in the supernatants was not taken into account by the above approximation used to calculate the percentage of L chains that were free. To demonstrate that this omission was not significant, we measured IgA in the supernatants of cultures from one spleen. Including the IgA

measurement in the calculation for this spleen, only reduced the calculated percentage of L chains that were free by 3%. Thus our approximation of the percentage of L chains in the free form appears valid.

We wished to confirm the finding of a λ predominance of free L chains in an additional system. Reagents that identify κ or λ free L chains but not L chains associated with H chains would be ideal for this purpose. Unfortunately, we determined that available antisera were not adequate to distinguish free L chains from those that were associated (data not shown). Thus, to assess the isotype (κ or λ) of the free L chains secreted, we cultured splenocytes with media, PWM, or LPS and then assayed the concentrated supernatant as follows. First, we determined the κ/λ ratio for exclusively the L chains associated with H chains. For this measurement, we used anti-IgG or anti-IgM capture antibody directly adsorbed to the plate followed by anti-κ or anti-λ alkaline phosphatase conjugates. We also assayed for total L chains (free L chains plus L chains associated with H chains) using anti-κ or anti-λ capture antibodies. These two assays differed only in whether free L chains were detected. Thus differences in the κ/λ ratio determined by these two assays would be attributed to the κ and λ composition of the free L chains.

A κ/λ ratio of one or more was demonstrated for IgG- and IgM-associated L chains whether measured in stimulated or unstimulated cultured supernatants (Fig. 4, *panels B* and *C*). However, when both L chains phenotypes (free and H chain associated) were assayed, λ chains far exceeded κ chains in the unstimulated cultures (Fig. 4*A*). Thus free L chains (the predominant form of L chains in unstimulated cultures) are predominantly λ whereas L chains associated with H chains are predominantly κ.

DISCUSSION

We examined κ and λ L chain concentrations secreted in vitro in cultures of human splenocytes. The expected κ L chain predominance of total Ig (L chains associated with Ig H chains) was noted. The IgG and IgM associated κ L chains were predominant in unstimulated B cell cultures as well as in cultures stimulated with PWM or LPS. However, in unstimulated cultures, free L chains were the predominant form of L chains detected. Furthermore, the free L chains secreted were predominantly λ isotype. Thus in the absence of Ig H chain expression, B cells secrete predominantly λ L chains.

Others have reported that unstimulated human B cell cultures produce excess free L chains although the κ/λ ratio of the free L chains was not determined (9). However, an altered κ/λ L chain ratio in the absence of H chains has been demonstrated for murine hybridomas (10). In the previously reported studies, the frequency of λ chains produced by the hybridomas was much higher for secreted free L chains than what was observed for secreted L chains associated with H chains. But these studies were not conducted with normal B cells and thus the significance was uncertain.

In normal B cells it is believed that the order of gene rearrangements is H chain Ig genes first, then κ L chain genes, and finally λ L chain genes if κ rearrangement is non-productive (1, 2). After the rearrangement, assembly of the chains occurs and then secretion. During this process, the occurrence of preferential association between H chains and κ L chains would be consistent with our data. If H chains preferentially associate with κ chains, then the κ predominance of IgG and IgM Ig would result. In contrast, for L chains secreted unassociated with H chains (free L chains), the κ/λ ratio would be determined by other mechanisms. Preferential H and L chain associations have been demonstrated in human cells by Kubagawa et al. (11) in examining pre-B cell leukemias. In addition, Ag-specific preferential association has been shown for streptococcal group A carbohydrate antibody in a murine system (12). In these studies a particular κ-chain idiotype was shown to be preferentially paired with H chains of the IgG3 subclass.

We have recently extended these findings of Ag-specific L chains associations with IgG antibody to the human response. We demonstrated that the κ/λ ratio of human serum antibody directed to certain capsular polysaccharides differs from the κ/λ ratio of total Ig (13). For example, the κ/λ ratio of antibody directed to *Haemophilus influenzae* type b and *Neisseria meningitidis* type c capsular polysaccharide were significantly increased as compared to the κ/λ of total Ig. In addition, we have shown that the magnitude of the human IgG response to *H. influenzae* type b capsular polysaccharide is significantly correlated with an individuals' κ/λ ratio of that response (14). Thus regulation of the IgG antibody response to certain polysaccharides is correlated with mechanisms associated with L chain selection. Inasmuch as little is known regarding the regulation of either of these events, it is difficult to speculate on a proposed mechanism for this correlation. However, it is possible that a subset of B cells responsive to polysaccharides could also be a subset more likely to secrete κ L chains.

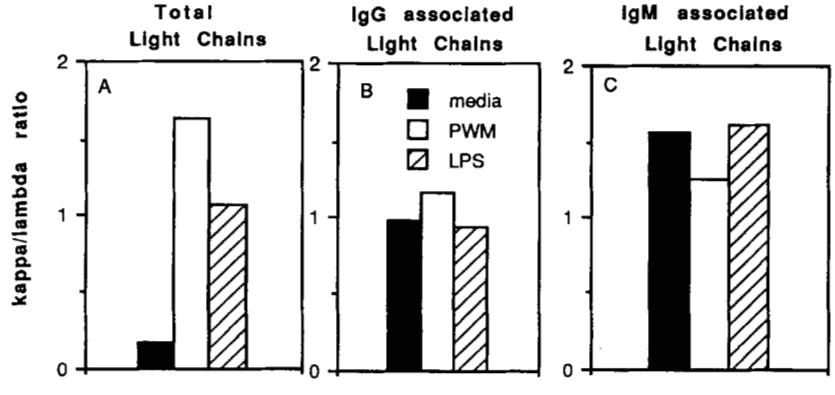

Figure 4. The κ/λ L chain ratios of total L chains, IgG associated L chains, and IgM associated L chains. *A*, Mean κ/λ ratio of total L chains (free L chains plus L chains associated with H chain) in supernatant of two cultures of splenocytes stimulated with PWM, LPS, or unstimulated. *B*, Mean κ/λ ratio of IgG associated L chains in the same supernatants. *C*, Mean κ/λ ratio of IgM associated L chains in the same supernatants.

One could speculate that this subset represents a more mature B cell subset as both the ability to respond to polysaccharides and the ability to produce κ antibodies are related to maturation (14). Alternatively, B cells with κ L chains on their surface may have increased affinity for polysaccharides and then become activated. Finally, evidence exists that T cells (or their products) influence both polysaccharide responses as well as light chain selection (6, 15). Thus a common T cell influence could be responsible for the correlation between the IgG response and the κ/λ ratio of the response.

These speculations on the mechanism of light selection may also apply to differences noted between LPS- and PWM-stimulated cultures. LPS is thought to stimulate relatively immature B cell subsets and requires less T cell help to secrete Ig as compared to PWM (8). We noted that the geometric mean κ/λ ratio of 1.3 for PWM-stimulated cultures was higher than the ratio of 0.9 obtained for LPS stimulated cultures ($p = 0.03$). Although both were significantly higher than the κ/λ ratio of unstimulated B cell cultures, differences in either the B cells stimulated or T cell help might be responsible for this effect. We suggest that the κ/λ ratio could be affected in vitro by the differences in B cell subset or the T cell subset that LPS and PWM elicit.

In summary, these studies of in vitro L chain secretion by human splenocytes demonstrate that H chain expression affects L chain selection. We propose that these findings support preferential H and L chain association in human B cells. Further studies on the regulation of L chain selection may elucidate the mechanism of preferential association and perhaps explain Ag-specific differences in the κ/λ ratio of human responses.

Acknowledgments. We thank Dr. Robert Shamberger and Dr. Harry Kozakewich for their cooperation in these studies.

REFERENCES

1. **Korsmeyer, S. J., P. A. Hieter, J. V. Ravetech, D. G. Poplack, T. A. Waldman, and P. Leder.** 1980. Developmental hierarchy of immunoglobulin gene rearrangements in human leukemic pre-B-cells. *Proc. Natl. Acad. Sci. USA 78:7096.*
2. **Heiter, P. A., S. J. Korsmeyer, T. A. Waldman, and P. Leder.** 1981. Human immunoglobulin kappa light-chain genes are deleted or rearranged in lambda-producing B-cells. *Nature 290:368.*
3. **Pepe, V. H., G. E. Sonenshein, M. I. Yoshimura, and M. J. Shulman.** 1986. Gene transfer of immunoglobulin light chain restores heavy chain secretion. *J. Immunol. 136:2367.*
4. **Edelman, G. M.** 1973. Antibody structure and molecular immunology. *Science 180:830.*
5. **Byum, A.** 1968. Isolation of mononuclear cells and granulocytes from human blood. *Scand. J. Clin. Lab. Invest. 21:S97.*
6. **Ambrosino, D. M., N. R. Delaney, and R. C. Shamberger.** 1990. Human polysaccharide-specific B cells are responsive to pokeweed mitogen and IL-6. *J. Immunol. 144:1221.*
7. **Siber, G. R., and D. M. Ambrosino.** 1986. Heavy and light chain restriction of human antibodies to polysaccharide antigens. *Clinical Use of Intravenous Immunoglobulins.* U. E. Nydegger, and A. Morell, eds. Academic Press, London, p. 47.
8. **Mayumi, M., T. Kuritani, H. Kubagawa, and M. D. Cooper.** 1983. IgG subclass expression by human B lymphocytes and plasma cells. *J. Immunol. 130:671.*
9. **Smith, J. L., and A. C. Hamam-Harris.** 1981. Induction of balanced immunoglobulin chain synthesis in free light chain-producing lymphocytes by mitogen stimulation. *J. Immunol. 126:1848.*
10. **Holmberg, D., I. Lundvist, L. Forni, F. Ivars, and A. Coutinho.** 1985. Absence of immunoglobulin heavy chain expression results in altered κ/λ light chain ratios. *J. Mol. Cell. Immunol. 2:51.*
11. **Kabagawa, H., M. Mayumi, W. M. Crist, and M. D. Cooper.** 1983. Immunoglobulin heavy-chain switching in pre-B leukemias. *Nature 301:340.*
12. **Fulton, R. J., M. H. Nahm, and J. M. Davie.** 1983. Monoclonal antibodies to streptococcal group A carbohydrate. *J. Immunol. 131:1326.*
13. **Ambrosino, D. M., V. A. Barrus, G. G. Delange, and G. R. Siber.** 1986. Correlation of Km(1) immunoglobulin allotype with anti-polysaccharide antibodies in Caucasian adults. *J. Clin. Invest. 78:361.*
14. **Ambrosino, D. M., W. Grief, C. Thompson, and G. R. Siber.** 1990. κ and λ light chain composition of antibody to the capsular polysaccharide of *Haemophilus influenzae* type b. *J. Infect. Dis. 161:922.*
15. **Burkly, L. C., and H. H. Wortis.** 1985. T cell regulation on light chain expression: preferential enhancement of IgK production by T cells in the response to DNP. *J. Immunol. 135:1577.*