

HIGH FREQUENCY OF  $\lambda$  GENE ACTIVATION IN BONE  
MARROW PRE-B CELLS

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It is well known that in the mouse most B cells carry  $\kappa$  light (L) chain, whereas a small percentage of B cells carry  $\lambda$  L chain (1-3). Assuming that the L chain commitment is a random process, the low frequency of  $\lambda$  producers could be attributable to the lower number of the variable region (V) genes of  $\lambda$  chain ( $V_\lambda$ ) than that of  $V_\kappa$  (4). Recent studies on the L chain gene organization, however, suggest the existence of a more complicated process than the simple probabilistic process in L chain expression. In both human and mouse, it was shown that almost all  $\lambda$  producers possess rearranged  $\kappa$  genes, while a majority of  $\kappa$  producers retained  $\lambda$  genes unrearranged (5, 6). These findings led to a model postulating that rearrangement of  $\lambda$  gene in pre-B cells started only after nonproductive rearrangements in both  $\kappa$  alleles (an ordered model of the L chain rearrangement). In this model, the frequency of B cells bearing activated  $\lambda$  gene depends primarily on the frequency of the pre-B cells that have committed the nonproductive rearrangements in both  $\kappa$  alleles (the frequency of  $\kappa^-/\kappa^-$ ). Since the frequency of  $\kappa^-/\kappa^-$  type pre-B cells was recently suggested to be more than 25% (7, 8), the frequency of  $\lambda$  gene activation could be much higher than the frequency of  $\lambda$ -bearing B cells in peripheral lymphoid organs. In order to attain an exact estimation of the frequency of the  $\lambda$  gene activation in pre-B cells, it is necessary to follow the course of B cell differentiation from pre-B cells.

Recently, in vitro system has been developed in which normal pre-B cells from various sources differentiate into functional B cells (9, 10). In this paper, we took advantage of such a culture system and attempted to investigate the frequency of  $\lambda$  expression in the immunoglobulin (Ig)-producing cells that have been generated in vitro from bone marrow pre-B cells.

**Materials and Methods**

*Animals.* C57BL/6 and BALB/c mice were bred and maintained in our animal colony. SJL and SJA/9 mice were kindly donated by Dr. K. Okumura (University of Tokyo).

*Cells and Proteins.* TEPC183 ( $\mu\kappa$ ), X63 ( $\gamma_1\kappa$ ), As79 ( $\gamma_{2b}\kappa$ ), 2b-7 ( $\gamma_{2b}\kappa$ ), J606 ( $\gamma_3\kappa$ ), and HOPC1 ( $\gamma_{2a}\lambda_1$ ) myeloma cells, and 267 ( $\mu\lambda_1$ ) hybridoma cells were kind gifts from Dr. K. Rajewsky (Cologne University) and from Dr. M. White-Scharf and Dr. T. Imanishi-Kari (Massachusetts Institute of Technology). K3 ( $\mu\kappa$ ) hybridoma cell was produced in our laboratory. The purification of proteins from ascites fluid was as described previously

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(11). Isolation of the light chains from 267 and X63 proteins was done according to the method described by Garvey et al. (12).

*Antisera.* The preparation of rabbit anti-mouse Ig serum was as described previously (13). Rabbit anti-mouse  $\lambda$  chain serum was prepared by immunizing two rabbits first with a subcutaneous injection of 1 mg of 267 protein in Freund's complete adjuvant followed by intravenous repetitive boosting with 50  $\mu$ g of isolated  $\lambda$  chain at 6-wk intervals. The sera were collected 10 d after each boosting, examined for the activity to develop protein A (Pharmacia Fine Chemicals, Uppsala, Sweden) plaque-forming cells (PFC) produced by HOPC1 cells, and the sera showing good activity were pooled. Resulting serum was rendered  $\lambda$  specific by absorbing sequentially with TEPC183, X63, and J606 proteins coupled to Sepharose 4B (Pharmacia). Rabbit anti-mouse  $\kappa$  chain serum was prepared according to the schedule for preparing anti- $\lambda$  chain serum by using X63 protein and its  $\kappa$  chain as antigens. The activity for developing protein A PFC produced by 2b-7 cells was checked in each sera and the sera showing good activity were pooled. The serum was rendered  $\kappa$  specific by absorbing sequentially with 267 and HOPC1 proteins coupled to Sepharose 4B. Goat anti-TEPC183 anti-serum was affinity purified on a 267-coupled Sepharose 4B column and used as anti- $\mu$  antibody.

*Cell Preparation and Culture.* The preparation of spleen and bone marrow cells, the treatment of spleen cells by anti-Thy-1.2 monoclonal antibody (clone F7D5, Olac Ltd. Oxon, UK) and complement, and the removal of surface  $\mu$ -bearing cells ( $\mu^+$  cells) by panning on anti- $\mu$  coated petri dish were as described in our previous reports (11, 14). RPMI 1640 (Flow Laboratories, McLean, VA)-based medium and nutritional cocktail were as described previously (11) except for the lot of fetal calf serum (Lot No. 2Lo13; M.A. Bioproducts, Walkersville, MD). Lipopolysaccharide (LPS: *Escherichia coli* 055:B5, Difco Laboratories, Inc., Detroit, MI) and an autoreactive T cell line, a subline derived from a BALB/c T cell line reactive to trinitrophenylated keyhole limpet hemocyanin (TNP-KLH) and established according to the method of Kimoto and Fathman (15), were used as polyclonal B cell activators. If the culture had to be maintained more than 4 d, 50  $\mu$ l of nutritional cocktail was given every day, starting on day 3 of culture.

*Reverse Plaque Assay.* Total Ig-,  $\lambda$ -, and  $\kappa$ -producing cells were measured by the reverse plaque assay using protein A coupled sheep erythrocytes as targets (16). Each assay involved the control assay in which PFC was developed using the same dilution of de complemented normal rabbit serum. The number of PFC developed by normal rabbit serum was always 0.1–0.2% of  $\kappa$ -PFC. These values were subtracted from each value before calculating the mean.

## Results

*Specificity of Antisera for Developing Protein A PFC.* Since the specificity of anti- $\lambda$  serum used in this study is critical for the correct interpretation of the following results, all antisera used in this study were characterized by using a panel of well-characterized cultured myeloma and hybridoma cells. 500 cells of each type were assayed for protein A PFC in Cunningham's chambers using either anti-mouse Ig serum, anti- $\lambda$  serum, or anti- $\kappa$  serum. As shown in Table I, both anti- $\lambda$  and anti- $\kappa$  sera were highly specific for developing protein A PFC produced by  $\kappa$ - or  $\lambda$ -producing cells.

*$\lambda/\kappa$  Ratio in Ig-Producing Cells Generated by Polyclonal Stimulation.* The previous studies on  $\lambda$  chain expression in spleen B cells using immunofluorescence staining demonstrated that 3–5% of total B cells carry  $\lambda$  chain (1–3). In order to investigate whether this ratio remains unchanged after polyclonal stimulation of B cells,  $5 \times 10^4$  BALB/c spleen B cells were stimulated polyclonally by either LPS or the autoreactive helper T cell line. As shown in Table II (Expt. 1), the  $\lambda/\kappa$  ratios obtained after either stimulation are almost the same as that shown in the previous reports using immunofluorescence staining (1–3), suggesting that

these two kinds of polyclonal B cell stimulations do not cause a significant change of  $\lambda/\kappa$  ratio during the culture.

Next we investigated the frequencies of  $\kappa$ - and  $\lambda$ -producing cells in the LPS-stimulated spleen cells from SJL and SJA/9 mice, since it was shown by Geckler et al. (17) and Conter and Eisen (18) that the serum concentration of  $\lambda$ -bearing Ig was low in these strains of mice. As shown in Table II (Expt. 2), LPS induced  $\lambda$  production in both SJL and SJA/9 spleen cells were six- to sevenfold lower than that in BALB/c spleen cells.

*Generation of  $\lambda$ -Producers at a High Frequency in the Culture Where the Differentiation from Pre-B Cells to Ig-Producing Cells Occurs.* Bone marrow cells ( $5 \times 10^5$ ) deprived of  $s\mu^+$  cells or unselected spleen cells ( $5 \times 10^5$ ) as the control were cultured in the presence of LPS. Various days after culture, the cells were harvested and assayed for both  $\kappa$ - and  $\lambda$ -producing cells, and the results are shown in Table III. In the spleen cell culture, LPS-induced Ig production reached the peak on day 5, and  $\lambda/\kappa$  ratios were  $\sim 5\%$  during day 4 to 8 of the culture. In contrast, in the culture of  $s\mu^-$  bone marrow cells, almost no Ig production was observed until day 5, indicating the lack of mature LPS-reactive B cells at the beginning of the culture. A considerable number of Ig-producing cells were observed on day 6, and the response reached the peak on day 9. To our surprise, the  $\lambda/\kappa$  ratio in

TABLE I  
Specificity of Antisera for Developing Protein A Hemolytic Plaques\*

| Cells   | Class                  | PFC/500 cells |                |                 |
|---------|------------------------|---------------|----------------|-----------------|
|         |                        | Anti-Ig       | Anti- $\kappa$ | Anti- $\lambda$ |
| 267     | $\mu\lambda_1$         | 168           | 0              | 164             |
| HOPC1   | $\gamma_{2a}\lambda_1$ | 96            | 0              | 92              |
| K3      | $\mu\kappa$            | 242           | 280            | 0               |
| TEPC183 | $\mu\kappa$            | 46            | 53             | 0               |
| X63     | $\gamma_1\kappa$       | 141           | 176            | 0               |
| 2b-7    | $\gamma_{2b}\kappa$    | 160           | 201            | 0               |
| J606    | $\gamma_{3\kappa}$     | 182           | 164            | 0               |

\* Plaques were counted after incubation for 3 h at 37°C.

TABLE II  
 $\kappa$ - and  $\lambda$ -Producing Cells Induced in Spleen Cells by Polyclonal B Cell Activators

| Experiment     | Mouse  | Stimulations             | $\kappa$ -PFC* | $\lambda$ -PFC | $\lambda/\kappa$ (%) <sup>‡</sup> |
|----------------|--------|--------------------------|----------------|----------------|-----------------------------------|
| 1 <sup>§</sup> | BALB/c | LPS                      | 8,190          | 410            | 5.0                               |
|                |        | Autoreactive T cell line | 10,080         | 490            | 4.9                               |
| 2 <sup>¶</sup> | BALB/c | LPS                      | 112,500        | 5,400          | 4.8                               |
|                | SJL    | LPS                      | 218,000        | 1,750          | 0.8                               |
|                | SJA    | LPS                      | 191,000        | 1,340          | 0.7                               |

\* PFC/culture. Arithmetic mean of triplicate culture.

<sup>‡</sup>  $\lambda$ -PFC/ $\kappa$ -PFC  $\times 100$ .

<sup>§</sup> Anti-Thy-1.2 plus complement-treated BALB/c spleen cells ( $5 \times 10^4$ ) were cultured for 5 d with 50  $\mu$ g/ml of LPS or  $10^4$  cells of the autoreactive T cell line in 0.2 ml medium.

<sup>¶</sup> Spleen cells ( $5 \times 10^6$ ) were cultured for 5 d with 50  $\mu$ g/ml of LPS in 1 ml medium.

TABLE III  
*λ*- and *κ*-Light Chain Expression in LPS-Induced Ig-Producing Cells from Spleen Cells and Bone Marrow Pre-B Cells\*

| Days after culture | Spleen cells               |               |                                      | Bone Marrow pre-B cells |               |                         |
|--------------------|----------------------------|---------------|--------------------------------------|-------------------------|---------------|-------------------------|
|                    | <i>κ</i> -PFC <sup>‡</sup> | <i>λ</i> -PFC | <i>λ</i> / <i>κ</i> (%) <sup>§</sup> | <i>κ</i> -PFC           | <i>λ</i> -PFC | <i>λ</i> / <i>κ</i> (%) |
| 4                  | 64,000                     | 3,000         | 4.8                                  | 40                      | 0             | 0                       |
| 5                  | 124,000                    | 6,080         | 4.9                                  | 200                     | 10            | 5.0                     |
| 6                  | 99,200                     | 2,500         | 6.6                                  | 2,510                   | 400           | 16.0                    |
| 7                  | 21,600                     | 1,060         | 4.9                                  | 12,500                  | 2,320         | 18.6                    |
| 8                  | 10,500                     | 390           | 3.7                                  | 17,300                  | 4,050         | 23.4                    |
| 9                  |                            | ND            |                                      | 55,360                  | 17,900        | 32.3                    |
| 10                 |                            | ND            |                                      | 32,900                  | 13,100        | 39.8                    |
| 11                 |                            | ND            |                                      | 21,900                  | 8,680         | 39.6                    |

\* Untreated spleen cells ( $5 \times 10^5$ ) or bone marrow cells that were deprived of  $s\mu$ -bearing cells by panning ( $5 \times 10^5$ ) were cultured with 50  $\mu\text{g/ml}$  LPS in 1 ml of culture medium. ND, not done.

<sup>‡</sup> PFC/culture. Arithmetic mean of triplicate culture.

<sup>§</sup>  $\lambda\text{-PFC}/\kappa\text{-PFC} \times 100$ .

this culture was high, as high as 16% from the first day when a measurable response was obtained, and increased up to nearly 40% as the culture proceeded.

### Discussion

The major finding of this study is that the  $\lambda/\kappa$  ratio in Ig-producing cells that have been generated from bone marrow pre-B cells *in vitro* was three- to eightfold higher than that in Ig-producing cells generated from spleen B cells cultured in the same condition (16–40% vs. 5%). As clearly demonstrated in Table I, it is unlikely that this result was due to the cross-reactivity of our anti- $\lambda$  serum. Thus, for our result to be compatible with the previous reports showing that  $\lambda/\kappa$  ratio was ~5% in immature as well as mature B cell populations (1–3), it is necessary to postulate either that the frequency of  $\lambda$  gene activation is ~5% but our culture condition has the effect of increasing the  $\lambda/\kappa$  ratio up to 16–40%, or that the  $\lambda$  gene is activated at such a high rate as 16–40% but a large proportion of  $\lambda$ -bearing B cells were prohibited from entering the functional pool by a rapid *in vivo* selection.

Very recently, Burkly et al. (19) showed that dinitrophenylated (DNP) LPS induced a higher  $\lambda_1$ -bearing anti-DNP antibody than did DNP-KLH. The high frequency of  $\lambda$  producers generated in the pre-B cell culture, however, may not be due to the effect of LPS, since in spleen B cells, the proportion of  $\lambda$  producers induced by LPS did not exceed the level induced by helper T cell line (Table II, Expt. 1). Nevertheless, it is difficult to rule out the first possibility, since all culture conditions are more or less selective, and it is plausible to think that the frequency of  $\lambda$  gene activation is determined by the frequency of  $V_\lambda$  in total  $V_L$  genes.

On the other hand, the ordered model of L chain rearrangement which postulates that almost all  $\lambda$  producers originate from pre-B cells of  $\kappa^-/\kappa^-$  genotype, can easily explain the high frequency of  $\lambda$  gene activation in pre-B cells. Although it is unknown what proportion of  $\kappa^-/\kappa^-$  pre-B cells differentiate into functional B cells, the recent studies in which the frequency of  $\kappa^-/\kappa^-$  genotype

in pre-B cells was suggested to be more than 25% (7, 8) give an upper limit of the frequency of  $\lambda$  gene activation. Our result (in Table III) was consistent with this value of the upper limit.

With such a high frequency of  $\lambda$  gene activation in pre-B cells, one has to speculate that in our culture condition all newly arising B cells differentiated into Ig-producing cells without in vivo selection possibly working on the Ig receptor of some structural properties. In SJL mice a regulation that is likely to be connected with the structural properties of SJL  $\lambda_1$  constant region ( $C_{\lambda 1}$ ), namely that  $C_{\lambda 1}$  of SJL mice differ from that of other normal strains by one amino acid substitution, was recently demonstrated (3, 20, 21). If true, it is of interest to think that the similar mechanism could also work against V regions with some abnormalities (e.g. unstable  $V_H$ - $V_L$  combination). Since the high frequency of  $\lambda$  producers in LPS-stimulated pre-B cell culture was unchanged after making hybridomas from the cells of this culture (unpublished observation), further analysis of protein structures isolated from such  $\lambda$ -producing hybridomas is awaited.

### Summary

The frequency of  $\lambda$  light chain (L) producing cells in immunoglobulin-producing cells that have been generated in vitro from bone marrow pre-B cells was investigated. The frequency of  $\lambda$ -producing cells obtained in such a culture was three- to eightfold higher than that observed in the culture of mature spleen B cells. These results suggest that the activation of  $\lambda$  gene at pre-B cell stage occurs far more frequently than the frequency presumed from the percentage of  $\lambda$ -bearing cells in mature B cells.

The phenomenon analyzed here was first found by S. Nishikawa in collaboration with Drs. T. Takemori, M. Siekevitz, and A. Radbruch of Dr. K. Rajewsky's laboratory in Cologne.

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