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# OCCURRENCE OF SURFACE IgM, IgD, AND FREE LIGHT CHAINS ON HUMAN LYMPHOCYTES\*

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Considerable evidence has accumulated indicating that the leukemic lymphocytes of patients with chronic lymphatic leukemia (CLL) carry surface immunoglobulin (Ig) of a single light- and heavy-chain class and that the heavy chain is usually IgM (1-3). This has been taken as evidence of the monoclonality of the proliferating cells. Recently it has been reported that IgD represents the surface-Ig of a significant number of normal human peripheral blood lymphocytes (4, 5). Of special significance has been the study of Rowe et al. (6) demonstrating that most normal lymphocytes bearing IgM also have IgD on their surface. It appeared of special interest to study IgD in the monoclonal CLL situation and determine its relationship to the usual surface IgM that is present. The findings indicate that the majority of CLL cases have leukemic cells with IgD as well as IgM on their surface. In addition, free light chains were frequently found.

## Materials and Methods

**Anti-Immunoglobulin Antisera.**—Antisera against isolated IgM, IgG, IgA, IgD, Fab of fraction II human  $\gamma$ -globulin (Fr II), and kappa and lambda Bence Jones proteins were raised in New Zealand Red rabbits. For immunofluorescence, all antisera conjugated with tetramethylrhodamine isothiocyanate according to Amante et al. (7) were absorbed with an  $\alpha$ -globulinemic serum bound to the Sepharose 4B gel activated by cyanogen bromide. The anti- $\mu$  antiserum (Rho-anti- $\mu$ ) was further absorbed with Sepharose-bound Fr II, the anti- $\gamma$  (Rho-anti- $\gamma$ ) with Sepharose-bound IgM, IgA, Bence Jones kappa and lambda proteins, the anti- $\alpha$  (Rho-anti- $\alpha$ ) with Sepharose-bound Fr II, IgM, Bence Jones kappa and lambda proteins, and the anti- $\delta$  (Rho-anti- $\delta$ ) with Sepharose-bound Fr II, IgM lambda and IgA lambda myeloma proteins. The anti(Fab)- $\gamma$ -serum (Rho-anti-Fab) was not absorbed further. Antiserum specificities were ascertained by double diffusion and direct hemagglutination. The Rho-anti- $\mu$ , Rho-anti- $\gamma$ , Rho-anti- $\alpha$ , and Rho-anti- $\delta$  gave a single precipitin line with normal human sera. The Rho-anti- $\mu$  agglutinated only IgM-coated red cells, the Rho-anti- $\gamma$  only IgG-coated red cells, the Rho-anti- $\alpha$  only IgA-coated red cells, and the Rho-anti- $\delta$  only IgD-coated red cells. The Rho-anti Fab had activities against both kappa and lambda determinants. When the Rho-anti-Fab was absorbed with Sepharose-bound IgG lambda, or IgG kappa, it was specific for kappa or lambda determinants. For hemagglutination inhibition, the anti- $\mu$  antiserum after absorption with Fr II agglutinated IgM-coated red cells and this agglutination inhibited only by either IgM kappa or

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IgM lambda proteins. The anti- $\delta$  antiserum was absorbed with Fr II and a normal serum with low levels of IgD. After absorption, it was specific for  $\delta$ -determinants only. Similar absorptions and specificity experiments were done with the anti- $\gamma$  and anti- $\alpha$  antisera. Anti-kappa Bence Jones antisera were absorbed with purified pooled whole human  $\gamma$ -globulin or human sera. After this absorption, the antisera were specific for free kappa chain only. Similar absorptions were done with the anti-lambda antisera. An anti-Fr II light-chain antiserum (anti-Fr II-L) was a generous gift from Dr. W. Epstein of the University of California at San Francisco. This antiserum was used after absorption with whole myeloma proteins and a normal human serum.

**Lymphocyte Separation.**—Mononuclear cells were isolated from the peripheral blood of chronic lymphocytic leukemia patients by Ficoll-Hypaque discontinuous gradients. The cells were washed six times with 50 ml phosphate-buffered saline (PBS). For hemagglutination inhibition, cells were suspended in the concentration of  $1-2 \times 10^8$  cells/ml. For immunofluorescence,  $0.5-1 \times 10^7$  cells/ml suspensions were made. Viability of all lymphocyte preparations was greater than 95% by trypan blue exclusion.

**Immunofluorescence.**—Immunofluorescent staining of lymphocyte surface Ig was performed as described by Pernis et al. (8). For each preparation, 100–400 cells were counted. Antisera were routinely centrifuged to remove aggregates before use. Absorption of the Rho-anti- $\mu$  by Sepharose-linked IgM, the Rho-anti- $\gamma$  by Sepharose-bound IgG (purified from Fr II by DEAE column chromatography), the Rho-anti- $\alpha$  by Sepharose-bound IgA, the Rho-anti-Fab by Sepharose-bound IgG and the Rho-anti- $\delta$  by an isolated IgD myeloma protein removed the staining activities of these antisera. In these experiments, the blocking proteins used were different from the immunizing proteins.

**Hemagglutination and Hemagglutination Inhibition.**—Hemagglutination and hemagglutination inhibition experiments were carried out as described previously (9). Protein A and B proteins were coated onto human red cells by the bisdiazotized benzidine method (BDB). A lymphocyte suspension was incubated with an equal volume of appropriately diluted antisera for 45 min and after centrifugation, 0.1 ml of the supernate was used to test its ability to agglutinate indicator red cells. More than 90–95% lymphocytes were viable after absorption. By comparison of the inhibition efficiency of the cell suspensions in serial dilutions and known amounts of inhibitory proteins, the gross amount of surface Ig was estimated.

Initially, considerable difficulty was encountered in applying hemagglutination techniques to estimate lymphocyte surface IgM. This was overcome by applying antisera of highly limited specificities for certain regions of the  $\mu$  chain. Details of this method will be described elsewhere.

## RESULTS

**Surface Ig of CLL Lymphocytes.**—Both IgM and IgD were found on the surface of CLL lymphocytes as shown by representative cases A, B, C, and D (Table I). The Rho-anti-Fab antiserum measured the total percentage of

TABLE I  
Lymphocyte Surface Ig of Representative CLL Cases

| Patient | Immunofluorescence |     |     |       |        |
|---------|--------------------|-----|-----|-------|--------|
|         | IgM                | IgD | Fab | Kappa | Lambda |
|         | %                  | %   | %   | %     | %      |
| A       | 88                 | 89  | 94  | 6     | 78     |
| B       | 73                 | 67  | 74  | 70    | 91     |
| C       | 12                 | 87  | 93  | 6     | 91     |
| D       | 80                 | 3   | 86  | 83    | 91     |

bearing cells in these patients since the antiserum had both anti-kappa and anti-lambda activities. In the first two instances (A and B) the majority of the Ig-bearing lymphocytes were stained with both the Rho-anti- $\mu$  and the Rho-anti- $\delta$ . Since these antisera were highly specific for  $\mu$  or  $\delta$ -determinants it was apparent from the percentages stained that the vast majority of cells bore both IgM and IgD on their surface. In the third instance (C) IgM was not detectable by fluorescence except in a small number of cells which represented at least in part normal B cells in the sample. No significant number of  $\gamma$ - and  $\alpha$ -bearing lymphocytes were found in these cases. A total of 15 CLL cases were studied and in 10 a high percentage of the cells showed IgD; in most instances IgM was found as well on the same high percentage of cells. Four cases showed IgM and not IgD; three showed IgD and not IgM; one showed low percentages of IgM, IgD, and Fab. In all the cases studied there was predominantly one class of light chains on lymphocytes of an individual CLL patient. The pattern of staining by these three rhodamine-conjugated antisera was finely granular. However, the intensities of lymphocyte staining by these antisera varied among different patients. In general, when IgM and IgD were both present, Rho-anti- $\mu$  and Rho-anti- $\delta$  stained with similar intensities.

Considerable experience has been gained recently in the use of hemagglutination inhibition techniques for obtaining semiquantitative information on surface Igs on lymphocytes. This method has the advantage of applying antisera of clearly defined specificity as determined by experiments such as those shown in Table II. In three cases in Table I, IgD was readily detected on the lymphocyte surface in quantities that clearly related the IgD to the leukemic cells. IgM was detectable similarly in cases A and B but barely so in case C. No IgG or IgA were detected in any of the cases. In case C, lambda determinants were found and no kappa determinants were detected. In all respects the results were similar to those obtained by fluorescence.

*Detection of Free Light Chains on CLL Lymphocytes.*—It is known that anti- $\kappa$  can be made to light chains that detect determinants that are hidden when light chains exist in combination with heavy chains in whole proteins (11). Such antisera were prepared to both kappa and lambda Bence-Jones proteins and after absorption showed such "free chain" specificity. Table II illustrates

TABLE II  
*Specificity of Anti-Kappa Chain Serum Absorbed with Normal Human Serum*

|                                   | Inhibitor protein concentration (mg/ml) |     |      |       |        |         |
|-----------------------------------|-----------------------------------------|-----|------|-------|--------|---------|
|                                   | 1                                       | 0.1 | 0.01 | 0.001 | 0.0001 | 0.00001 |
| Kappa Bence Jones                 | 0                                       | 0   | 0    | 0     | 0      | 2       |
| Lambda Bence Jones                | 2                                       | 2   | 2    | 2     | 2      | 2       |
| IgG K                             | tr                                      | 2   | 2    | 2     | 2      | 2       |
| IgM K                             | 2                                       | 2   | 2    | 2     | 2      | 2       |
| Normal human serum (diluted 1/10) | 2                                       | 2   | 2    | 2     | 2      | 2       |

Coating protein: Kappa Bence Jones protein.

this point. Only kappa Bence-Jones proteins inhibited the agglutination of the kappa protein coated red cells by the antiserum whereas whole IgG kappa, IgM kappa, and normal human sera did not inhibit the agglutination. Similar specificity only for free lambda chains was also obtained from anti-lambda Bence-Jones antisera. The anti-Fr II-L had specificities for both free kappa and lambda chains.

When these hemagglutination inhibition systems were applied to the quantitation of free light chains on CLL lymphocytes, lymphocytes of seven CLL patients were found to have readily detectable amounts of free light chain on their surface (Table III). The range of free light chains was between 125-1,000 ng per  $10^5$  cells. Free light chains were found on both kappa- and lambda-

TABLE III  
*Detection of Free Light Chains on CLL Lymphocyte Surface*

| Patient | Class of light chain | Free light chain* |
|---------|----------------------|-------------------|
|         |                      | ng/ $10^5$ cells  |
| E       | Kappa                | 1,000             |
| F       | Kappa                | 500               |
| G       | Kappa                | 250               |
| H       | Kappa                | 125               |
| I       | Kappa                | 125               |
| J       | Lambda               | 2*                |
| K       | Lambda               | 2                 |

\* Approximate values from the semiquantitative hemagglutination inhibition technique.

bearing CLL lymphocytes. Determination of the total light chains on the same CLL cells by the usual anti-kappa and anti-lambda antisera with specificities against light chain determinants shared by both free chains and those in combination with heavy chains, indicated that the free light chains represented a significant portion of the total. In only one of eight cases studied were the free light chains undetectable.

#### DISCUSSION

In view of the monoclonal nature of the proliferating CLL cells with only a single class of light chains, these cells are proving extremely useful for studying the characteristics of a homogeneous cell population. Their B-cell nature and high percentages of Ig-bearing cells is now firmly established in almost all cases (3). The findings that the majority of CLL lymphocytes carry both IgG and IgM on their surface offers strong supportive evidence for the conclusion of Rowe et al. (6) from their studies on normal human lymphocytes. Pernicelli and Rowe have also observed IgD on leukemic lymphocytes recently.<sup>1</sup> Evidence was obtained in the present study as well as the previous one (5) that the

<sup>1</sup> Personal communication.

and IgD are formed by the cells themselves and are not taken up from the serum. The single light-chain type for each leukemia, with both kappa and lambda represented, offers strong evidence on this point. It also indicates that both the IgD and IgM have similar light chains. It appears probable that they also possess identical V regions and should share the same idiotypic specificity. This is currently under investigation.

The significance of the free light chains on the leukemia cells remains to be determined. The use of antisera specific for the free chains determinants offered a simple procedure for their detection. It remains to be determined whether similar amounts are found on normal B cells. More quantitative procedures for the light chains as well as for IgM and IgD are required to answer questions such as this. Evidence for some free surface light chains has been obtained in single instances previously by other methods (11, 12).

15 CLL cases were investigated in the present study and it was apparent from both fluorescence and hemagglutination inhibition studies that IgD was present on the surface of the leukemia cells in most but not all cases. In three instances the IgD was present in a high percentage of cells without detectable IgM. The major question concerning the presence of both Igs on these B cells is their relationship with respect to receptor function. This question remains unanswered but it appears clear that IgD must play a key role.

#### SUMMARY

An analysis was made of the immunoglobulin surface markers of the cells of patients with chronic lymphatic leukemia (CLL) in view of previous evidence of their monoclonal B-cell character. The simultaneous presence of IgM and IgD on the surface of the majority of lymphocytes was demonstrated by both immunofluorescence and hemagglutination inhibition in most cases. However, cases were observed with surface IgM without IgD as well as cases with IgD without IgM. IgG and IgA were absent. Studies of the light chains indicated only a single class in a given case. In addition to bound light chains, free light chains were readily demonstrated in most cases through the use of antisera specific for "free chain" determinants. It thus appeared that there are three major types of surface Ig on CLL lymphocytes, IgM, IgD, and free light chains.

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