sue-specific mRNAs to be sequenced from at least some and possibly all tissues, and the conservation of sequence through evolution allows mRNAs from other species to be sequenced without cloning. In addition, the transcript can be translated in vitro, thereby allowing the intact protein or any desired segment to be produced.

REFERENCES AND NOTES

1. C. Wong *et al.*, *Nature* **330**, 384 (1987); D. R. Engelke, P. A. Hoener, F. S. Collins, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 544 (1988).
2. E. S. Stoflet, D. D. Koeberl, G. Sarkar, S. S. Sommer, *Science* **239**, 491 (1988).
3. T. Maniatis, E. F. Fritsch, J. Sambrook, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982).
4. Since oligonucleotides accumulate rapidly when GAWTS is used, it is important to have informative names. The following nomenclature readily allows the determinaton of (i) the size of the amplified fragment, (ii) the appropriateness of any combination of oligonucleotides, and (iii) the origin and direction of the sequence generated. It is of the form: G(O)-(I-L)R(C)-SD, where G is gene abbreviation, O is organism, I is identifier (one or more) for the noncomplementary 5′ bases, L is length of the noncomplementary bases, R is region of the gene, C is location of the 5′ complementary base, S is total size, and D is 5′ to 3′ direction of the oligonucleotide. The region of the gene (R) is abbreviated by 5′, the region upstream of the gene; by E followed by exon number; by I followed by intron number; or by 3′, the region downstream of the gene. The direction of the oligonucleotide is either U, upstream, or D, downstream. If a transcript has been defined, D is the sense direction and U is the antisense direction. Otherwise the directions can be arbitrarily defined. Thus BP(Hs)-(T7-29)E5(1453)-44U is an oligonucleotide specific for the blue pigment gene of *Homo sapiens*, which has a T7 promoter (includes a 6-base clamping sequence of the 5′ end) of 29 bp. It is complementary to a sequence in exon 5 that begins at base 1453. The oligonucleotide is 44 nucleotides and it heads upstream relative to the blue pigment mRNA. If there is no chance of confusion with other sequences, the name can be abbreviated in routine use by omitting one or more of the understood designations such as "BP," "(Hs)," and "(T7-29)." As another example, F9(Hs)-5′(−120)-15D is an oligonucleotide specific for human factor IX. It is complementary to a 15-base sequence 5′ to the human factor IX gene that begins at base −120. The oligonucleotide is 15 nucleotides and the sequence heads downstream relative to in vivo transcription [G. Sarkar and S. S. Sommer, *Nucleic Acids Res.* **16**, 5197 (1988)].
5. S. Yoshitake, B. G. Schach, D. C. Foster, E. W. Davie, K. Kurachi, *Biochemistry* **24**, 3736 (1985).
6. J. Nathans *et al.*, *Science* **232**, 193 (1986).
7. S. C. M. Kwok, F. D. Ledley, A. G. DiLella, K. J. H. Robson, S. L. C. Woo, *Biochemistry* **24**, 556 (1985).
8. A. G. DiLella, S. C. M. Kwok, F. D. Ledley, J. Marvit, S. L. C. Woo, *ibid.* **25**, 743 (1986).
9. B. Grima *et al.*, *Nature* **326**, 707 (1987).
10. K. L. O'Malley *et al.*, *Biochemistry* **26**, 6910 (1987).
11. R. K. Saiki *et al.*, *Science* **239**, 487 (1988).
12. D. J. G. Rees, C. R. Rizza, G. G. Brownlee, *Nature* **316**, 643 (1985).
13. M. Kozak, *Mol. Cell. Biol.* **8**, 2737 (1988).
14. J. Pelletier and N. Sonenberg, *Cell* **40**, 515 (1985).
15. G. Sarkar and S. S. Sommer, unpublished observations.
16. W.-H. Li, M. Tarimura, P. M. Sharp, *J. Mol. Evol.* **25**, 330 (1987).
17. D. D. Koeberl, C. D. K. Bottema, J.-M. B. Buerstedde, S. S. Sommer, manuscript in preparation.
18. RAWTS is a four-step procedure. (i) *First strand cDNA synthesis:* 20 λ of 50 μg of heat-denatured total RNA or mRNA per milliliter, 50 m*M* tris-HCl (*p*H 8.3), 8 m*M* $MgCl_2$, 30 m*M* KCl, 1 m*M* dithiothreitol (DTT), 2 m*M* each of dATP, dCTP, dGTP, dTTP, oligo(dT) 12–18 (50 μg/ml), RNasin (1000 U/ml), and AMV reverse transcriptase (1000 U/ml) were incubated at 42°C for 1 hour followed by 65°C for 10 min. Subsequently, 30 λ of H_2O was added for a final volume of 50 λ. (ii) *PCR:* 1 λ of the above sample was added to 40 λ of 50 m*M* KCl, 10 m*M* tris-HCl (*p*H 8.3), 1.0 to 2.5 m*M* $MgCl_2$ (empirically determined for each set of primers), 0.01% (w/v) gelatin, 200 μ*M* each of dNTP, 1 μ*M* of each primer (Perkin-Elmer Cetus protocol). After 10 min at 94°C, 1 U of *Taq* polymerase was added, and 40 cycles of PCR were performed (annealing: 2 min at 50°C; elongation: 3 min at 72°C; denaturation: 1 min at 94°C) with the Perkin-Elmer Cetus automated thermal cycler. One primer included a T7 promoter as previously described (*2*). (iii) *Trancription:* After a final 10-min elongation, 3 λ of the amplified material was added to 17 λ of the RNA transcription mixture. The final mixture contains 40 m*M* tris-HCl (*p*H 7.5), 6 m*M* $MgCl_2$, 2 m*M* spermidine, 10 m*M* sodium chloride, 0.5 m*M* of the four ribonucleoside triphosphates, RNasin (1 U/λ), 10 m*M* DTT, 10 U of T7 RNA polymerase, and diethylpyrocarbonate-treated H_2O. Samples were incubated for 1 hour at 37°C, and the reaction was stopped by heating at 65°C for 10 min. (iv) *Sequencing:* 2 λ of the transcription reaction was added to 10 λ of annealing buffer containing the end-labeled reverse transcriptase primer. Annealing and sequencing were performed essentially as described, but note that [δ-^{32}P]ATP is the correct donor for end-labeling (*2*).
19. K. Katayama *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 4990 (1979).

20. Supported in part by grant 5-647 from the March of Dimes–Birth Defect Foundation. We thank E. J. W. Bowie for his support and encouragement.

28 October 1988; accepted 7 February 1989

Expression of High-Affinity Binding of Human Immunoglobulin E by Transfected Cells

LARRY MILLER, ULRICH BLANK, HENRY METZGER, JEAN-PIERRE KINET

The receptor with high affinity for immunoglobulin E (IgE) on mast cells and basophils is critical in initiating allergic reactions. It is composed of an IgE-binding α subunit, a β subunit, and two γ subunits. The human α subunit was expressed on transfected cells in the presence of rat β and γ subunits or in the presence of the γ subunit alone. The IgE binding properties of the expressed human α were characteristic of receptors on normal human cells. These results now permit a systematic analysis of human IgE binding and a search for therapeutically useful inhibitors of that binding.

THE HIGH-AFFINITY RECEPTOR FOR IgE ($Fc_\epsilon RI$) plays a central role in initiating allergic reactions by coupling the presence of allergen to the release of mediators by mast cells and basophils. Using basophilic leukemia (RBL) cells, we showed that $Fc_\epsilon RI$ in the rat is a tetramer consisting of a single IgE-binding α subunit, a single β subunit, and two disulfide-linked γ subunits (*1*). Little is known about the human $Fc_\epsilon RI$ because obtaining sufficient numbers of normal mast cells and basophils is impractical and because no stable cell lines expressing high-affinity receptors for human IgE exist as yet. Knowledge about the structure and function of the human receptor will be critical for determining whether this protein can serve as an appropriate target for new therapeutic approaches to allergy, for example, by inhibiting IgE binding or the earliest steps in receptor activation.

Our aim therefore was to obtain expression of human IgE binding by transfected cells. The cDNA for the IgE-binding human α subunit has been cloned but could not be expressed in transfection experiments (*2, 3*). Possibly, as with the homologous rat α, expression requires cotransfection with the cDNA for the β (*4*) and γ subunits (*5*). However, the library from which the cDNA for human α was obtained has not yet yielded cDNA clones for human β and γ (*6*), and indeed the cell line used to construct that library fails to bind IgE (*7*). We therefore attempted to express human IgE binding by cotransfecting cells with the cDNA for the human α subunit together with the cDNA for rat β or γ subunits, or both.

A 907-bp Sfa NI fragment was excised from the cDNA that codes for the α subunit of the human IgE receptor and subcloned into the vector pSVL for expression by transfected COS 7 cells. This fragment includes the entire sequence coding for α plus a minimal amount of 5′ and 3′ untranslated sequence. The pSVL–human α cDNA was cotransfected along with separate pSVL plasmids containing cDNAs coding for the rat β and γ subunits. When these transfectants [$\alpha_H(\beta\gamma)_R$ cells] were reacted with mouse monoclonal IgE (specific for dinitrophenyl), 5% to 7% of cells formed rosettes with trinitrophenylated red blood cells. Cells cotransfected with α_H and β_R failed to form rosettes, but $\alpha_H\gamma_R$ transfectants showed rosetting (Table 1). The latter result was unexpected because we had found earli-

Section on Chemical Immunology, Arthritis and Rheumatism Branch, National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health, Bethesda, MD 20892.

er that expression of transfected cDNA for the rat α required cotransfection with both rat β and γ (*5*) (Table 1).

Both $\alpha_H(\beta\gamma)_R$ and $\alpha_H\gamma_R$ transfectants failed to form rosettes when non–antigen-specific rat or human IgE was added in excess before the cells were incubated with the hapten-specific mouse IgE. These results are consistent with the species specificity for IgE expressed by the human high-affinity receptor (*8*).

Binding of human IgE to hybrid human-rat receptors was also studied directly by adding ^{125}I-labeled human IgE to the transfected cells. By dividing the total number of receptors binding ^{125}I-labeled IgE by the number of rosette-forming cells in each transfected cell preparation, one could determine that cells transfected with either $\alpha_H(\beta\gamma)_R$ or $\alpha_H\gamma_R$ expressed as many as 1×10^6 to 1.5×10^6 cell surface receptors. This calculation is based on the assumption that rosetting cells were the only ones to express any receptors (an assumption that may not be literally true). Binding was completely inhibited by the presence of unlabeled human or rodent IgE. No direct IgE binding was observed with cells transfected with only α_H or with $\alpha_H\beta_R$, thus confirming the results obtained in the rosetting assay.

The association rate constant (k_{+1}) for the binding of human IgE was estimated by monitoring the initial rate of interaction of ^{125}I-labeled human IgE with the transfected cells. The rate of association with receptors on $\alpha_H(\beta\gamma)_R$ cells and $\alpha_H\gamma_R$ cells was similar (Fig. 1A). Using the equation $k_{+1} = V_0/[\text{IgE}]_0[R]_0$, where $[\text{IgE}]_0$, $[R]_0$, and V_0 refer to the starting concentrations and to the initial rate, we estimated $k_{+1} \pm$ SD to be $(0.9 \pm 0.07) \times 10^5 M^{-1}\ s^{-1}$ at 25°C.

We also measured the dissociation rate constant (k_{-1}). The dissociation rate of bound IgE from both transfected $\alpha_H(\beta\gamma)_R$ and $\alpha_H\gamma_R$ cells resembled the dissociation rate of ^{125}I-labeled mouse IgE from the endogenous receptors on RBL cells (Fig. 1B). From the slopes of the plots shown in Fig. 1B, we estimated $k_{-1} \pm$ SD to be $(1 \pm 0.17) \times 10^{-5}\ s^{-1}$ under the conditions used. The equilibrium association constant (k_{+1}/k_{-1}) calculated from our data is in the range $0.83 \times 10^{10} M^{-1}$ to $1.0 \times 10^{10} M^{-1}$. Together these data show that transfected hybrid human-rat receptors exhibit binding properties characteristic of high-affinity Fc_ϵ receptors (*8, 9*) (Table 1).

The studies discussed so far indicated that cotransfection with the cDNAs for rat subunits was required for expression of the cDNA for α_H, but did not prove that the human and rat subunits were actually expressed as a hybrid oligomeric receptor. To determine this, we first incubated cells with excess ^{125}I-labeled human IgE, then washed and solubilized them. The centrifuged extract was immunoprecipitated with a monoclonal antibody (JRK) that reacts specifically with the β chain of the rat high-affinity receptor (*10*). In cells transfected with $\alpha_H(\beta\gamma)_R$ we were able to precipitate at most 20% of receptor-bound IgE with the antibody to the β chain (anti-β), whereas in control experiments run in parallel on RBL cell extracts, 80% of receptor-bound ^{125}I-labeled mouse IgE was precipitated.

We performed various experiments to discover the basis for this difference. It was possible that an excess of β chains, or some other change in the transfected cells, prevented effective immunoprecipitation of chimeric receptors. We explored this possibility

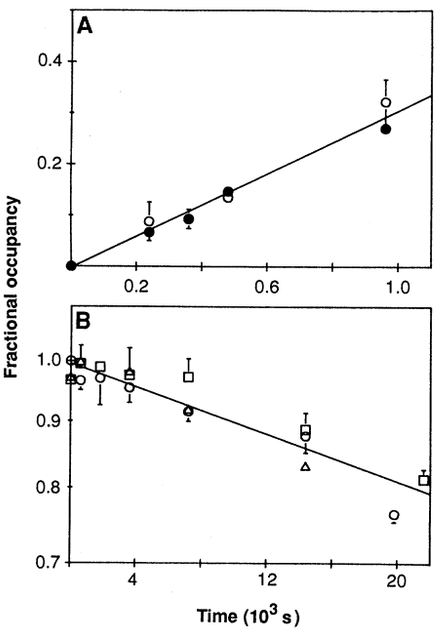

Fig. 1. Association and dissociation kinetics for the interaction of IgE with transfected cells and RBL cells. (**A**) Rate of association of human IgE with $\alpha_H(\beta\gamma)_R$ and $\alpha_H\gamma_R$ cells. The binding ability and initial rate of association of ^{125}I-labeled human IgE (2.5 μg/ml) with $\alpha_H(\beta\gamma)_R$ (●) and $\alpha_H\gamma_R$ (○) cells (5×10^6 cells per milliliter) were measured at 25°C (*9*). Values are the means of triplicate measurements, and error bars represent standard deviations of individual data points. Data points correspond to the amount of IgE bound at each time, divided by the amount bound after 2 hours of incubation. Nonspecific binding of IgE, estimated by incubating cells with unlabeled human IgE (200 μg/ml) and then with ^{125}I-labeled IgE, was subtracted from total binding at each time point. The line drawn through the data represents a first-order regression analysis for the entire data set. (**B**) Dissociation of receptor-bound IgE from transfected cells and RBL cells. The dissociation reaction was measured basically as described by Kulczycki and Metzger (*9*). $\alpha_H(\beta\gamma)_R$ (○) or $\alpha_H\gamma_R$ (□) cells (5×10^6 cells per milliliter) were loaded with ^{125}I-labeled human IgE (2.5 μg/ml) for 1 hour at 25°C. RBL cells (△) were loaded with ^{125}I-labeled mouse IgE (2.5 μg/ml). At various times, cells were pelleted through phthalate oil (*17*), and the cell-bound IgE was assayed. Nonspecific binding of IgE, estimated by addition of unlabeled human or mouse IgE to the cells before addition of the appropriate labeled IgE, represented 0.1% to 0.3% of the added ^{125}I-labeled IgE and was subtracted from the total binding. Data points represent the mean of at least duplicate measurements. Error bars represent standard deviations of individual data points. Error bars for the RBL cell data have been omitted for clarity but were comparable to those presented for the transfected cells. The line drawn through the data points represents a first-order regression analysis for the entire data set.

Table 1. Binding characteristics of transfected and endogenous receptors. COS 7 cells were transfected with 80 μg of DNA by electroporation with a Bio-Rad Gene Pulser apparatus by the method recommended by the manufacturer or with 40 μg of DNA by a standard $CaPO_4$ precipitation technique (*15*). After 48 hours, transfected cells were sensitized with anti-dinitrophenyl IgE as described previously (*5*) and rosetted with ox red blood cells modified with 2,4,6-trinitrobenzene sulfonic acid as described by Rittenberg *et al.* (*16*). All of the binding constant data are for human IgE except for experiments with RBL cells in which rat IgE was used. The values are for binding at 25°C. Values in the table for k_{+1} and k_{-1} represent the mean of calculated values from two to four experiments ± SD. For K_a, the SD was propagated from the standard deviations for the ratio of the two rate constants (k_{+1}/k_{-1}). Abbreviations: H, human; R, rat; Ro, rodent; a dash indicates that no binding was observed; NA, not assayed.

Cell	IgE binding	Percentage of rosetting cells	Binding constants		
			k_{+1} ($10^5 M^{-1}\ s^{-1}$)	k_{-1} ($10^{-5}\ s^{-1}$)	K_a ($10^{10} M^{-1}$)
α_H	—				
$\alpha_H\beta_R$	—				
$\alpha_H\gamma_R$	H, Ro	5–8	0.8 ± 0.07	0.9 ± 0.13	0.9 ± 0.17
$\alpha_H(\beta\gamma)_R$	H, Ro	5–7	0.9 ± 0.09	1.2 ± 0.17	0.8 ± 0.17
α_R	—				
$(\alpha\beta)_R$	—				
$(\alpha\gamma)_R$	—				
$(\alpha\beta\gamma)_R$	R o	5–7			
RBL*	R o	100	0.5 ± 0.08	0.5 ± 0.12	1.0 ± 0.28
Human basophils†	H, Ro	NA	0.9 ± 0.37	2.4 ± 0.36	0.4 ± 0.44

*Data for RBL are from (*9*). †Data for human basophils (*8*) have been corrected for temperature with the same temperature factor determined in (*9*).

by performing immunoprecipitation experiments with mixtures of extracts from RBL and transfected cells, in which only one or the other cell type had been preloaded with ^{125}I-labeled IgE. The results showed that the presence of extract from COS 7 cells transfected with $\alpha_H(\beta\gamma)_R$ did not prevent or reduce the precipitation by anti-β of receptor-bound IgE derived from RBL cells (Fig. 2A), and the presence of extract from RBL cells did not enhance the precipitability of receptor-bound IgE in extracts from the

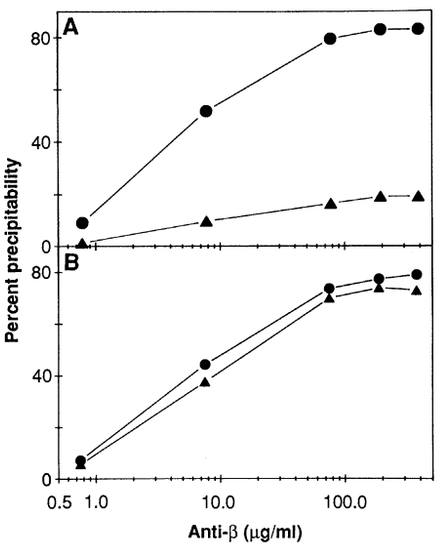

Fig. 2. Coimmunoprecipitation of receptor-bound IgE by anti-β. (**A**) Coimmunoprecipitation of receptor-bound IgE from mixtures of $\alpha_H(\beta\gamma)_R$ and RBL cells. (▲), Transfected COS cells (1×10^7 in 1 ml) were loaded with ^{125}I-labeled human IgE (5 μg/ml) for 1 hour at 25°C, washed in complete medium, mixed with 2×10^6 RBL cells loaded with unlabeled mouse IgE, washed again, and solubilized with 10 m*M* CHAPS (15 min on ice) (*18*). The extracts were centrifuged 15 min at 4°C in an Eppendorf microfuge and then reacted for 2 hours (4°C) with varying concentrations of monoclonal anti-β (JRK). After an additional 2-hour incubation with a fivefold molar excess of rabbit antibody directed against mouse IgG-Fc (Jackson Immunochemicals, West Grove, Pennsylvania), treated extracts were immunoprecipitated with Pansorbin (Calbiochem). Data points correspond to the fraction of receptor-bound IgE precipitated at each concentration of anti-β. (●) RBL cells (2×10^6 in 1 ml) were loaded with ^{125}I-labeled mouse IgE (5 μg/ml), mixed with transfected COS cells loaded with unlabeled human IgE, and treated as described above. (**B**) Coimmunoprecipitation of receptor-bound IgE from mixtures of COS cells transfected with $(\alpha\beta\gamma)_R$ and RBL cells. (▲) Transfected cells (1×10^7 in 1 ml) were loaded with ^{125}I-labeled mouse IgE (5 μg/ml), mixed with 2×10^6 RBL cells loaded with unlabeled mouse IgE, and treated as described in (A). (●) RBL cells (2×10^6 in 1 ml) were loaded with ^{125}I-labeled mouse IgE (5 μg/ml), mixed with 1×10^7 transfected cells loaded with unlabeled mouse IgE, and treated as described. Data are individual precipitations from single transfected cell preparations. Precipitation profiles from at least one additional preparation were comparable.

$\alpha_H(\beta\gamma)_R$ cells. That the transfection procedure itself is not the critical factor is shown by the data in Fig. 2B; that is, receptor-bound IgE in extracts from cells transfected with all three rat subunits, $(\alpha\beta\gamma)_R$, was as precipitable (Fig. 2B) as the analogous IgE bound to the endogenous receptors from RBL cells (Fig. 2B).

Oligomeric rat receptors $(\alpha\beta\gamma)_R$ are relatively unstable in the detergents required to solubilize them (*11, 12*). Chimeric receptors such as $\alpha_H(\beta\gamma)_R$ or $\alpha_H\gamma_R$ might be even more unstable. If so, one would anticipate that compared to native rat receptors, $\alpha_H\gamma_R$ would be poorly precipitable by anti-γ because β and γ always dissociate equivalently from $(\alpha\beta\gamma)_R$ (*10, 12*). However, IgE bound to cells transfected with $\alpha_H\gamma_R$ was as precipitable as that bound to $(\alpha\beta\gamma)_R$ (Fig. 3) and therefore appears to be as stable as the native rat receptor. Thus the poor precipitability by anti-β of ^{125}I-labeled IgE bound to cells transfected with $\alpha_H(\beta\gamma)_R$ is likely due to α_H being expressed as $\alpha_H(\beta\gamma)_R$ and $\alpha_H\gamma_R$ alternatively on such cells (*13*).

The unexpected finding that α_H can be expressed without β, whereas α_R—whose transmembrane segment in particular is substantially homologous to α_H—can only be expressed as $(\alpha\beta\gamma)_R$ (*5*), may prove useful. If analyzed in detail, it may provide further insights into how the subunits interact with each other to form the complete receptor.

With respect to the assembly of the receptor we note a curious finding. We recently detected a substantial homology between the putative transmembrane and immediately surrounding segment (but not elsewhere) of the γ chain of $Fc_\epsilon RI$ and the ζ (zeta) chain of the CD3 complex on T lymphocytes; 17 of 29 amino acids (49 of 87 nucleotides) are identical between the two and notably the transmembrane aspartic acid residue is preserved (*5, 14*). Like γ of $Fc_\epsilon RI$, two ζ chains are thought to be disulfide-linked to each other via transmembrane cysteines and appear to promote the expression of the other subunits of the CD3 complex. It would be interesting to explore whether, because of its homologous transmembrane segment, the ζ chain could substitute for γ in assisting the expression of the α chain of $Fc_\epsilon RI$.

From the perspective of allergic phenomena, the most important aspect of our experimental results is that the ability to express human IgE-binding receptors now permits the development of in vitro assay systems to screen for possible inhibitors of binding of human IgE. Production of a stably transfected cell line would be most useful for such studies, and we have isolated at least one such cloned line by transfecting CHO cells. However, surface expression of

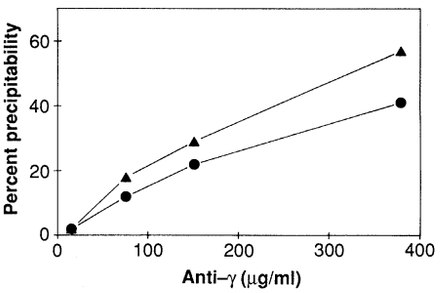

Fig. 3. Coimmunoprecipitation of receptor-bound IgE by anti-γ. $\alpha_H\gamma_R$ cells (1×10^7 in 1 ml) were loaded with ^{125}I-labeled human IgE (5 μg/ml) for 1 hour at 25°C and mixed with 0.75×10^6 RBL cells loaded with mouse IgE (5 μg/ml). A second set of cells were loaded and mixed at the same concentrations with ^{125}I-labeled mouse IgE for RBL and unlabeled human IgE for the transfected cells. Cell mixtures were washed and solubilized at 2×10^7 cells per milliliter as described in Fig. 2. Centrifuged extracts were reacted with a polyclonal antibody prepared against a synthetic peptide corresponding to residues 41 to 47 of the rat γ sequence (*5*). (▲) Coimmunoprecipitation of receptor-bound ^{125}I-labeled human IgE from $\alpha_H\gamma_R$ cells in presence of mouse IgE–loaded RBL cells. (●) Coimmunoprecipitation of receptor-bound ^{125}I-labeled mouse IgE in extracts from RBL cells in the presence of extracts of $\alpha_H\gamma_R$ cells loaded with unlabeled human IgE. The data points presented in this figure are single precipitation points from one transfected cell preparation.

receptors in this particular clone has been highly variable. The transient transfection system can be used for screening inhibitors on a laboratory scale and for studying site-specific mutants of receptor subunits. Mutagenesis of the α chain, for example, may help to establish points of contact between human IgE and α, thereby assisting in the rational design of inhibitors of binding.

REFERENCES AND NOTES

1. H. Metzger, J.-P. Kinet, R. Perez-Montfort, B. Rivnay, S. A. Wank, *Progress in Immunology* (Academic Press, Orlando, FL, 1983), vol. 5; H. Metzger, *et al.*, *Annu. Rev. Immunol.* **4**, 419 (1986).
2. A. Shimizu *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 1907 (1988).
3. J. Kochan, L. F. Pettine, J. Hakimi, K. Kishi, J.-P. Kinet, *Nucleic Acids Res.* **16**, 3584 (1988).
4. J.-P. Kinet *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 6483 (1988).
5. U. Blank *et al.*, *Nature* **337**, 187 (1989).
6. J. Kochan and J.-P. Kinet, unpublished observations.
7. T. Fukuda *et al.*, *Blood* **70**, 612 (1987).
8. T. Ishizaka *et al.*, *J. Immunol.* **134**, 532 (1985).
9. A. Kulczycki, Jr., and H. Metzger, *J. Exp. Med.* **140**, 1676 (1974).
10. J. Rivera *et al.*, *Mol. Immunol.* **25**, 647 (1988).
11. B. Rivnay, S. A. Wank, H. Metzger, *Biochemistry* **21**, 6922 (1982).
12. J.-P. Kinet, G. Alcaraz, A. Leonard, S. Wank, H. Metzger, *ibid.* **24**, 4117 (1985).
13. It is conceivable that COS 7 cells (a monkey kidney cell line) contain an endogenous β chain that we failed to detect by Northern blotting with our cDNA probe for rat β. However, this is highly unlikely since extensive blotting studies with that same probe on rat cell lines failed to detect β transcripts in any cells other than mast cells (*5*).

Therefore, on $\alpha_H\gamma_R$ cells the receptor is likely expressed as an oligomer of α and γ chains, whereas for the $\alpha_H(\beta\gamma)_R$ cells, only about 20% of their expressed receptors are of the $\alpha_H(\beta\gamma)_R$ type under the conditions of our transfection. Thus, the binding properties of the cells collected in Table 1 reflect the binding properties of the mixture of $\alpha_H(\beta\gamma)_R$ and $\alpha_H\gamma_R$.

14. A. M. Weissman *et al.*, *Science* **239**, 1018 (1988).
15. L. G. Davis *et al.*, *Basic Methods in Molecular Biology* (Elsevier, New York, 1986).
16. M. D. Rittenberg and K. L. Pratt, *Proc. Soc. Exp. Biol. Med.* **132**, 575 (1969).
17. D. M. Segal and E. Hurwitz, *J. Immunol.* **118**, 1338 (1977).

18. The numbers of RBL cells and transfected cells in the mixtures were adjusted so that each cell type would contribute an approximately equal number of receptors. After being washed, the mixed cells were suspended in the detergent to obtain a final concentration of 2×10^7 cells per milliliter. This is a lower concentration than we usually use because the COS cells are substantially larger than the RBL cells. An appropriate cell concentration is important in order to obtain both solubilization and a cell lipid/detergent ratio that will maintain the receptors' integrity (*11, 12*). Complete medium consisted of Dulbecco's modified Eagle's medium (Biofluids, Rockville, MD) to which had been added 17% fetal bovine serum (Gibco, Grand Island, NY) and 2.0 m*M* glutamine.

19. Supported by an NRC/NIH Associateship (L.M.) and a fellowship from the Deutsche Forschungsgemeinshaft (U.B.). The authors thank G. Poy for technical assistance.

6 December 1988; accepted 3 March 1989

Activation of an Excluded Immunoglobulin Allele in a Human B Lymphoma Cell Line

NEIL BERINSTEIN,* SHOSHANA LEVY, RONALD LEVY

Mature B cells that express surface immunoglobulin (Ig) are usually committed to their original Ig product. It was shown that such a cell can replace its light chain by rearranging and expressing a new light chain from the other allele. Anti-idiotype antibodies were used to isolate idiotypic variants from a surface $IgM^+\lambda^+$ human B cell tumor line. The variants expressed a new λ light chain. Both the original and the new λ transcripts were present in the variant cells, but only the new one was expressed as a protein on the cell surface. Therefore, although the cell exhibited allelic exclusion and had only one Ig receptor at a time, the commitment to a particular light chain gene was reversible.

FUNCTIONAL IG GENES ARE CREATED during B cell differentiation by the rearrangement of discrete germ line gene segments (*1*). Once formed, these rearranged Ig genes can be further altered during clonal evolution to give rise to a diverse repertoire of antibody-producing cells. Three different mechanisms have been described by which the combining site of the Ig product of a B cell may be modified. These include ongoing Ig gene rearrangement (*2*) and replacement of the variable (V) region of previously rearranged Ig genes (*3*) in pre–B cells and point mutation in B cells (*4*). We now report an additional genetic mechanism of variation occurring in a human B lymphoma cell line, the activation and expression of the other allele.

We isolated rare variants of a surface $IgM^+\lambda^+$ cell line derived from a patient with a diffuse large cell lymphoma (*5*) that had altered idiotypic structures. Three phenotypic groups of clonally related variants were isolated by two-color cell sorting. Variants showed either a complete loss of reactivity, a partial loss of reactivity, or varied reactivity with a panel of different anti-idiotype antibodies. To confirm that these variants were indeed unique, their Igs were examined by two-dimensional gel electrophoresis. A representative clone from each of the three groups expressed heavy chains that were indistinguishable, but light chains of slightly different apparent molecular size and isoelectric mobility (Fig. 1A). Variation in the λ light chain gene was also documented by DNA blot analysis of wild-type and variant DNA with a Cλ probe. The three variants shared a rearranged band not present in the DNA of the parental cell (Fig. 1B).

To understand the molecular basis for this light chain heterogeneity, we cloned and sequenced the V-region cDNA from the parental cell and from two idiotype-negative variants. A transcript (T1) was expressed by the parental cell that coded for an unusual λ light chain protein, in that position 88 contained aspartic acid rather than the invariant cysteine residue. An identical transcript was also found in each of the variants. However, the variant cells expressed, in addition, a second transcript (T2). The V and joining (J) regions of T1 and T2 were different (Fig. 2). In addition, the constant (C) region of T2 differed at amino acid 119 from the C region of T1 by one nucleotide of the 80 bases sequenced (CCA instead of CCG).

To determine whether a T2 was expressed in the parental cell, we made probes from V-region fragments unique to each transcript (indicated in Fig. 2). These probes distinguished between V regions when tested on phage DNA containing these genes. Messenger RNA from the parental cell and from three idiotype-negative clones was examined by RNA blot analysis with T1- and T2-specific probes and with a human actin control probe (Fig. 3). T1 was expressed by all three idiotype-negative clones, as well as by the parental cell. When corrected for differences in mRNA loaded on the gel, as indicated by the actin probe, no differences were seen in T1 expression in the variants as compared to the parental cell. In contrast, a T2 was expressed only by the idiotype-negative variants and not by the parental cell.

Further evidence that the variants were expressing a λ Ig transcript different from that of the parental cell was obtained by a serological analysis of the protein. Flow cytometry was performed on the parental cell and on variants C5 and C14 with monoclonal antibody 14 D1, which differentiates between λC region allotypes $Kern^-Oz^-$ and $Kern^-Oz^+$ (*6*). The two variants both reacted with 14D1, which indicated that they used λC region Oz^-, whereas the parental cells were unreactive (Fig. 4).

All idiotype-negative clones expressed T2 even though the variants were phenotypically distinct and produced light chain proteins that migrated differently on two-dimensional gels. We compared the sequences of T2 from idiotype-negative variants C14 and C5 (Fig. 2). The two clones had identical V, J, and C regions. The sequences differed from each other by seven nucleotides at the V-J junction, resulting in two amino acid differences between the two proteins. Except for this junctional difference, the V-region sequences of the T2 transcripts were identical. Likewise, V-region sequences of T1 transcripts from the parental cells, from an idiotype-positive clone, and from the two variants were all identical. Therefore, there was no evidence for somatic hypermutation in this cell line.

We have shown that a mature B cell can rearrange a second light gene at a different allele even though it had originally produced a functional surface Ig product. Although rearrangements often occur during B cell development on both Ig alleles, the expressed Ig protein is usually produced from only one allele (*7*). This phenomenon has been referred to as allelic exclusion (*8*). In

Department of Medicine, Stanford University Medical Center, Stanford, CA 94305.

*Present address: Toronto-Bayview Cancer Center and University of Toronto, 2075 Bayview Avenue, Toronto, Ontario M4N 3M5, Canada.