A Human Monoclonal Antibody to Insulin

AVI LIVNEH, HAVA AVRAHAM, DANA ELIAS, JOSEPH SACK, IRUN R. COHEN, AND ZELIG ESHHAR

SUMMARY
To elucidate the immune aspects of insulin-dependent diabetes mellitus (IDDM), we attempted to generate human monoclonal anti-insulin antibodies by fusing peripheral blood lymphocytes obtained from 10 insulin-treated IDDM patients with cells from a human lymphoblastoid cell line. Hybridomas that secreted immunoglobulins appeared in 9 of 400 wells. One of these hybridomas secreted anti-insulin antibody of the IgM class. The lymphocytic partner of this hybridoma was obtained from an IDDM patient who had undetectable levels of antibodies to insulin in his serum. Thus, by employing the hybridoma technique, it was possible to reveal the presence of insulin-sensitized B-lymphocytes in a patient who was serologically negative for anti-insulin antibodies. The monoclonal antibody recognized intact human insulin and insulins of other species, but not isolated A- and B-chains. This indicates that the antibody was functionally an autoantibody directed to an epitope formed by the native conformation of a highly conserved portion of the insulin molecule. This is the first report of a human hybridoma antibody to insulin. DIABETES 1986; 35:68–73.

The formation of anti-insulin antibodies in diabetes patients treated with insulin is well documented.[1] Insulin resistance, insulin allergy, and lipoatrophy are among the clinical problems attributed to anti-insulin antibodies.[1–4] Therefore, much attention has been focused on anti-insulin antibodies in diabetes. As homogeneous subpopulations of antibodies can provide us with information not revealed by heterogeneous antibodies, we set out to produce human anti-insulin antibodies as monoclonal antibodies using the hybridoma technique. This technique, first described by Köhler and Milstein,[5] has been further developed to obtain human monoclonal antibodies.[6] In this article, we report the isolation of an anti-insulin antibody-secreting hybridoma made by fusing human lymphoblastoid cells with peripheral blood lymphocytes of a patient with insulin-dependent diabetes mellitus (IDDM).

From the Departments of Internal Medicine (A.L.) and Pediatrics (J.S.), Sheba Medical Center, Tel-Hashomer; and the Departments of Cell Biology (A.L., D.E., I.R.C.) and Chemical Immunology (C.A., Z.E.), The Weizmann Institute of Science, Rehovot, Israel.
Address reprint requests to I. R. Cohen, M.D., Department of Cell Biology, The Weizmann Institute of Science, Rehovot, 76 100, Israel.
Received for publication 11 February 1985 and in revised form 26 June 1985.

MATERIALS AND METHODS

Human lymphoblastoid cell line. The ARH-77 cell line LICR/LON/HMy2[7] was kindly donated by Dr. M. J. O'Hare of the Ludwig Institute, London, United Kingdom. The cells were maintained in RPMI 1640 medium supplemented with 15% fetal calf serum (FCS; Biolab, Jerusalem, Israel), 1% nonessential amino acids, 100 U/ml penicillin, 100 μg/ml streptomycin, and 1 mM Hepes buffer solution. This supplemented medium will hereafter be designated complete medium. Before fusion, the cells were shown to be sensitive to aminopterine (0.176 μg/dl) and resistant to 8-azoguanine (20 μg/ml).

Human peripheral blood lymphocytes. Lymphocytes were obtained from IDDM patients. Twenty milliliters of whole blood was drawn and collected into a 50-ml plastic tube containing 250 U of heparin. Forty milliliters of blood diluted 50% (vol/vol) in RPMI 1640 was layered on a Ficoll-Hypaque (sp. gr. 1.1007) and centrifuged at 400 x *g* for 30 min at room temperature. The lymphocytes were collected, washed in RPMI 1640, and counted.

Hybridoma production and maintenance. Lymphoblastoid cells were mixed with an equal number of lymphocytes from each patient and washed in RPMI 1640. The pellet was resuspended in 2 ml of 42% (wt/vol in RPMI 1640) polyethylene glycol, mol. wt. 1500 (PEG; Serva, Heidelberg, FRG), and gently mixed for 2 min at 37°C. The PEG was then diluted by slowly adding RPMI 1640, 35 ml, at a gradually increasing rate for 15 min. The cells were centrifuged and resuspended (10^5 cells/ml) in complete medium to which was added hypoxanthine, aminopterine, and thymidine (HAT medium[8]) and plated by adding 1 ml of the suspension to each well of 24-

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/35/1/68/354470/35-1-68.pdf by guest on 11 September 2024

well tissue culture plates (Costar, Cambridge, Massachusetts). The cells were incubated (37°C, 6% CO_2) and fed weekly by changing about two-thirds of the medium to fresh HAT medium. Hybridoma growth was detected after 6–8 wk by a change in the color of the medium and by an increased number of cells. Hybridomas were transferred to 10-ml and later to 50-ml tissue cultured flasks (Nunc, Kamstrup, Denmark). To ensure monoclonality, cloning was done by limiting dilution of the hybrid cells. Wells displaying cell growth were analyzed for activity and recloned.

Solid-phase radioimmunoassay (RIA). The solid-phase RIA was performed as described.[9] Flexible polyvinyl chloride microtiter plates (Cook Labs., Alexandria, Virginia) were coated with test antigen or with control protein by adding 100 μl of 100 μg/ml of the material diluted in phosphate-buffered saline (PBS) for an incubation period of 1–2 h. All incubations in the RIA were done at room temperature. Wells were washed twice with 5% FCS (in PBS) and saturated with 5% FCS for 30 min. Fifty microliters of the test solution was added to the wells and incubated for 2–3 h, then washed twice with 5% FCS. Twenty-five microliters of ^{125}I-radiolabeled detecting reagent (10^5 cpm) was added for 2–3 h of incubation and the plates were washed four times with 5% FCS, dried, and the wells were cut and counted separately in a gamma counter. For isotype determination, an additional intermediate detecting step was included before the radioactive reagent: the addition of 35 μl of diluted rabbit anti-human subclass immunoglobulin antibodies for 2–3 h. The experiments were repeated four times. Standard deviations in all experiments were <12% of the mean.

The reagents that were used in the different assays are listed in Table 1. They were purchased or prepared as follows: Goat anti-human Fab was prepared by immunization of a goat with pepsin-treated[10] pure human immunoglobulin (Bio-Yeda, Rehovot, Israel). The immune goat serum was affinity purified on Sepharose columns to which human Fab was linked.[11] The antibody so obtained and protein-A (Pharmacia, Uppsala, Sweden) were radiolabeled using the chloramine-T technique.[12] Monocomponent porcine insulin (Nordisk, Gentofte, Denmark), monocomponent human synthetic insulin (Nordisk), bovine insulin (Sigma, St. Louis, Missouri), carboxymethylated A-chain of porcine insulin (Mann Research Laboratories, U.S.A.), bovine serum albumin (BSA, Sigma), and all rabbit anti-human subclass immunoglobulins (Bio-Yeda) were purchased. A mixture of A- and B-chains of porcine insulin was prepared as published[13] and kindly provided by Dr. Y. Shechter of the Department of Hormone Research at the Weizmann Institute. Fish insulin was a gift of Dr. Madar of the Department of Biochemistry of the Faculty of Agriculture of the Hebrew University, Rehovot, Israel.

Inhibition studies. To test the ability of porcine insulin and of a mixture of A- and B-chains of porcine insulin to inhibit the binding of the monoclonal anti-insulin antibody to porcine insulin, a solution of anti-insulin antibody diluted to 50% of its binding was incubated with different amounts (10^{-3}–10^2 μg/ml) of the inhibitors separately. After 1 h at 37°C and 24 h at 4°C, the residual activity of the antibody was determined in 50 μl of the test solution using the solid-phase RIA (Table 1, no. 6).

Affinity of binding. Competition between porcine ^{125}I-insulin and unlabeled porcine insulin was used to determine the affinity of the hybridoma antibody. Solutions, each containing

TABLE 1
Solid-phase radioimmunoassays

No.	Assay	Antigen	Tested solution	Intermediate detecting layer	Detecting reagent
1	Screening of hybridoma supernatants for anti-insulin antibodies	Porcine insulin	Different Hyb SN ARH SN	—	^{125}I-G anti-H Fab
2	Detection of anti-insulin antibodies in sera	Porcine insulin	Increasing dilutions of IDDM patient and normal human sera	—	^{125}I-G anti-H Fab
3	Determination of monoclonal anti-insulin antibody isotype	Porcine insulin	Hyb 10 SN	Rabbit anti-Ig chains: γ, μ, α, κ	^{125}I-PA
4	Determination of immunoglobulin chain types in hybridoma supernatants	Rabbit anti-Ig chains: γ, μ, α, κ	Hyb 10 SN	—	^{125}I-G anti-H Fab
5	Binding of monoclonal anti-insulin antibody to insulin and its subunits	Porcine insulin A-chain A- and B-chain	Hyb 10 SN	—	^{125}I-G anti-H Fab
6	Inhibition of monoclonal anti-insulin antibody by insulin and its subunits	Porcine insulin	Hyb 10 SN diluted and preincubated with increasing concentrations of porcine insulin A- and B-chains, medium	—	^{125}I-G anti-H Fab

Radioimmunoassays were done as described[9] by adding the test solution to the antigen and detecting its binding by ^{125}I-radiolabeled reagents. BSA served as the control protein for the antigens in all tests. Abbreviations are as follows: Hyb SN, hybridoma supernatant; IDDM, insulin-dependent diabetes mellitus; ARH, lymphoblastoid cell line used for fusion; ^{125}I-G anti-H Fab, ^{125}I-labeled goat anti-human Fab; and ^{125}I-PA, ^{125}I-labeled protein A.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/35/1/68/354470/35-1-68.pdf by guest on 11 September 2024

2.5 μg of the purified monoclonal antibody, ^{125}I insulin (10^4 cpm, ~2 ng), and unlabeled insulin in increasing amounts ($1–10^5$ ng), all dissolved in PBS (final volume 50 μl), were incubated at 4°C for 16 h. Twenty-five microliters of killed *Staphylococcus aureus* (Cowan I, Bio-Yeda) 10% (wt/vol in PBS), to which purified goat anti-human Fab antibodies had been linked, was added to each tube. After incubation for 2 h at room temperature on an Eppendorf mixer, the mixtures were centrifuged for 5 min using a Beckman Microfuge (Beckman Instruments, Fullerton, California). The pellet was washed twice in PBS and its radioactivity measured. The ratio of the bound to free insulin was calculated and plotted against the concentration of bound insulin, and the K-value of the reaction was determined according to Scatchard's equation.[24] Porcine insulin was iodinated by the chloramine-T technique. Goat anti-human Fab was linked to the fixed *S. aureus* bacteria (ratio 1:100 wt/wt) by an overnight incubation of the two reactants at 4°C. The monoclonal antibody was affinity purified as described for lipogenesis inhibition (see below). The antibody was used in this assay in a concentration that gave 50% of maximal insulin binding in preliminary assays.

Karyotype determination. Karyotype analysis was performed as described.[14] A suspension of 10^6 hybridoma cells in complete medium was incubated with 0.5 μg/ml of colchicine (Gibco, Grand Island, New York) for 1 h at 37°C and then centrifuged at 1000 cpm for 6 min. The supernatant was aspirated and swelling of the cells in the pellet was achieved by adding 1 ml of 75 mM KCl for 15 min at 37°C. The cells were fixed by methanol-glacial acetic acid and examined after being spread, dried, and stained.

Inhibition of insulin-induced lipogenesis. Lipogenesis was performed as described.[15] A suspension of rat adipocytes

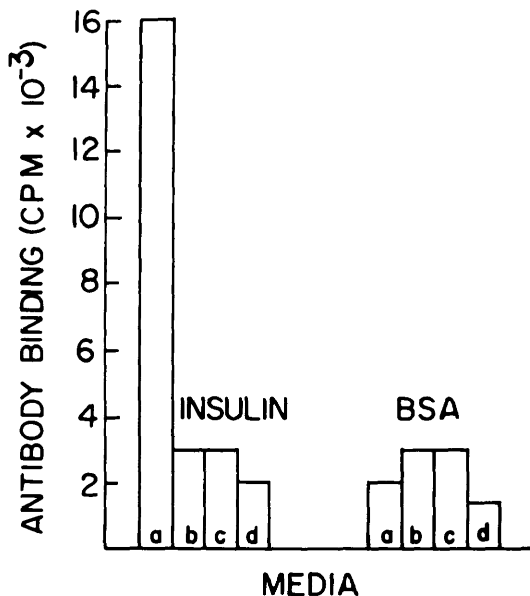

FIGURE 1. Antibodies to insulin in the supernatant of hybridoma 10. Antibodies were determined in undiluted culture media using the solid-phase RIA. Results of binding to insulin and to a control protein BSA of hybridoma 10 supernatant (A), an example of anti-insulin antibody-negative hybridoma supernatant (B), supernatant of lymphoblastoid cell line used for fusion (C), and medium (D) are given in counts per minute (cpm).

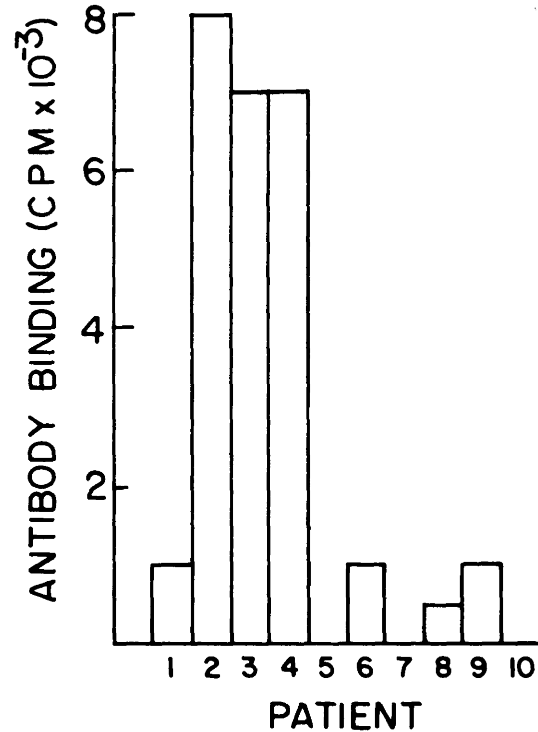

FIGURE 2. Antibodies to insulin in IDDM paitients' sera. Antibodies to insulin were determined in various dilutions of patients' sera using a solid-phase RIA (Table 1, no. 2). Results in counts per minute (cpm) are given for a serum dilution of 10^{-3} after subtraction of the counts per minute of nonspecific binding to BSA (2000 cpm). Negative sera also remained as such when lower dilutions ($10^{-1}–10^{-2}$) were examined (not shown).

(5×10^3 cells/ml) in Krebs-Ringer buffer containing 0.3% BSA was prepared as published[16] and added in aliquots of 0.5 ml to plastic vials, each containing 50 μl of D-[U-^{14}C]glucose (New England Nuclear, Boston, Massachusetts) and 10 μl of increasing concentrations of porcine insulin. Final concentration of the tracer was 0.2 mM and of insulin 0.02–10 ng/ml. The suspensions were incubated for 2 h at

TABLE 2
The immunoglobulin isotype of hybridoma anti-insulin antibody is IgM

Rabbit antibody to human Ig chain	Binding (cpm × 10^{-3}) to		
	Supernatant*		Anti-insulin Hyb 10 SX†
	Hyb 10 SN	ARH SN	
γ	22	15	2
μ	33	1.5	38
α	1	1.5	2
κ	22	9	22

Classes of the immunoglobulin (Ig) chains in the supernatants of hybridoma 10 (Hyb 10 SN) and of the lymphoblastoid cell line used for fusion (ARH SN) were determined by RIA using rabbit anti-human Ig chains. Results are given in counts per minute (cpm) after subtraction of the nonspecific binding to medium.

*ARH and hybridoma culture supernatants were tested on immobilized rabbit antibody to human Ig chains.

†Anti-insulin antibodies were immobilized on insulin bound to the assay plates and then tested with the rabbit antibody to human Ig chains.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/35/1/68/354470/35-1-68.pdf by guest on 11 September 2024

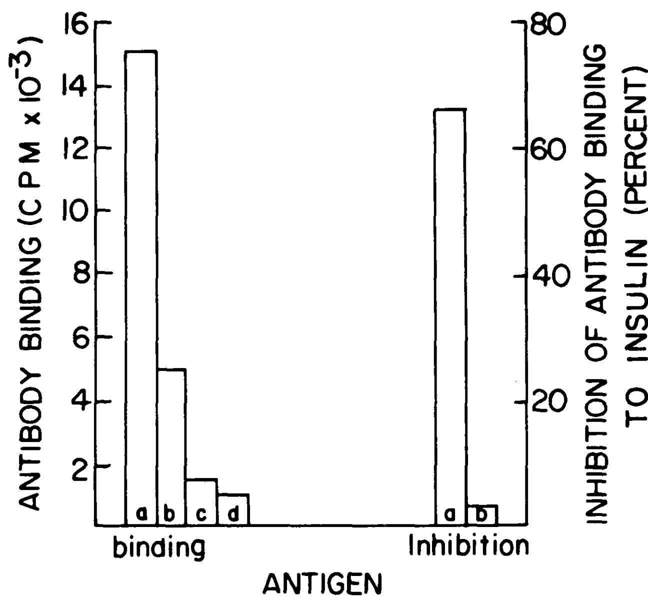

FIGURE 3. Binding and inhibition of monoclonal anti-insulin antibody. Binding of anti-insulin antibody to intact porcine insulin (A), separated A- and B-chains (B), A-chain (C), and BSA (D) was determined by solid-phase RIA (Table 1, no. 5). Results are given in counts per minute (cpm) for undiluted supernatants. Inhibition of anti-insulin antibody binding to insulin was calculated from the residual binding of anti-insulin antibody detected by solid-phase RIA after it had been incubated with various concentrations of insulin (A) and of A- and B-chains (B) (Table 1, no. 6).

37°C under an atmosphere of CO_2:O_2 (5:95 vol/vol). The incorporation of glucose into lipids was measured as counts per minute (cpm) after addition of 1 ml of toluene-based scintillation fluid to each vial. The ability of the monoclonal antibody to inhibit lipogenesis was tested at 0.2 ng/ml, the concentration of insulin giving 50% of maximal lipogenesis. Hybridoma 10 anti-insulin antibody was purified on an agarose column (5 × 0.5 cm) to which porcine insulin was immobilized as described.[17] The concentration of eluted anti-insulin antibody was determined by optical density at 280 nm and the purified antibody was added to the lipogenesis assay. Inhibition of lipogenesis was indicated by a reduction in counts per minute.

RESULTS

In 10 fusion experiments, a total of nine hybridomas were obtained–eight from one patient and one from another. The supernatant of the single hybridoma from patient 10, hybridoma no. 10, contained an immunoglobulin with insulin-binding capacity (Figure 1).

To test for monoclonality, the hybridoma cells were diluted serially. Growth was obtained in every well containing a concentration of 7.5 or more cells per well. Every well showing growth was positive for antibodies to insulin. A single well with a concentration of 7.5 cells was serially diluted a second time and, again, growth and antibodies to insulin were detectable only at a concentration of 7.5 cells or greater per well. Because of this requirement for a minimal cell density for growth, we cannot be certain that the hybridoma was monoclonal; nevertheless, it is highly likely that this was the case. The eight hybridomas that did not bind insulin were not studied.

TABLE 3
Binding of hybridoma 10 antibody to human and other insulins

Insulin	Hybridoma 10 antibody binding (cpm × 10^{-3})
Human	14
Porcine	15
Bovine	19
Fish	25
A- and B-chains	2
BSA	1

Insulins of various species, insulin A- and B-chains, or BSA were immobilized and assayed for their binding by hybridoma 10 anti-insulin antibodies.

Figure 2 shows the levels of anti-insulin antibodies in the sera of the 10 IDDM patients who were the donors of the lymphocytes for the fusion experiments. Note that patient no. 10, who was the donor of the lymphocytes from which the hybridoma-secreting, monoclonal anti-insulin antibody was derived, had undetectable levels of insulin antibodies. This patient was a 20-yr-old man with onset of IDDM at age 13 yr, who since then had been treated with highly purified NPH porcine insulin (Insulin Leo NPH, Nordisk). The IDDM was well controlled and the course of disease was uneventful.

Table 2 shows that the culture medium of hybridoma 10 contained μ- and γ-heavy chains and κ-light chains. The γ-chains could be assigned to the ARH lymphoblastoid cell line fusion partner, while the antibodies of hybridoma 10 that bound to immobilized insulin consisted of μ- and κ-chains. We concluded, therefore, that hybridoma 10 produced an anti-insulin monoclonal antibody of the IgM class. The hybridoma produced 2 μg/ml of IgM anti-insulin antibody at cessation of growth. The results reported here were obtained from a single batch of 300 ml of spent hybridoma medium.

Since the ARH cell line contains Epstein-Barr virus,[7] it was possible that the anti-insulin antibody-producing cell was a viral-transformed B-lymphocyte. However, karyotypic analysis revealed that the anti-insulin antibody-producing cell had about 100 chromosomes, indicating that it was a true somatic cell hybrid. The parental ARH lymphoblastoid cell line contained about 40 chromosomes (not shown).

The results illustrated in Figure 3 show that the monoclonal anti-insulin antibody bound to porcine insulin, but not to sep-

TABLE 4
Lipogenesis is inhibited by purified hybridoma 10 antibody

Hybridoma 10 antibody (μg/ml)	Insulin (ng/ml)	Lipogenesis		
		cpm	Percent maximum	Percent inhibition
0	10	3100	100	—
0	0.2	2300	50	0
8	0.2	2000	31	38
20	0.2	1700	13	75
0	0	1500	0	—

Affinity-purified hybridoma 10 anti-insulin antibody was added to the lipogenesis assay using 0.2 ng/ml insulin, the concentration of insulin giving 50% lipogenesis. Maximum lipogenesis was produced by 2–10 ng/ml of insulin. The percent inhibition was computed as: [1 − (test cpm − 1500/2300 − 1500)] × 100.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/35/1/68/354470/35-1-68.pdf by guest on 11 September 2024

arated A- and B-chains. An inhibition test (Figure 3) demonstrated that intact insulin in solution, but not separated A- and B-chains, inhibited the binding of the monoclonal anti-insulin antibody to porcine insulin in the solid-phase RIA. Thus, the epitope recognized by the anti-insulin antibody was formed by the insulin molecule in its native conformation.

Table 3 shows that hybridoma 10 anti-insulin antibodies bound to human, porcine, bovine, or fish intact insulins to a much greater degree than they bound to A- or B-chains or BSA. This indicates that the epitope recognized by this human monoclonal antibody was present in human insulin and highly conserved in evolution. The monoclonal antibody could thus be defined as an autoantibody to insulin.

To study functional aspects of the interaction between the hybridoma antibody and insulin, we investigated the effect of the antibody on the ability of insulin to stimulate lipogenesis in vitro. The monoclonal anti-insulin antibody was purified by affinity binding to insolubilized insulin and the eluted antibody was found to inhibit the lipogenic activity of insulin on adipocytes (Table 4). Thus, the binding of the hybridoma antibody to insulin affected its function.

Competition between ^{125}I-insulin and unlabeled insulin for binding to hybridoma 10 antibody and a Scatchard analysis of the results (Figure 4) led to an estimated affinity of $K = 10^4$ L/mol. *Staphylococcus aureus* goat anti-human Fab was used to precipitate the complex insulin antibody because the monoclonal antibody alone failed to precipitate insulin from solution.

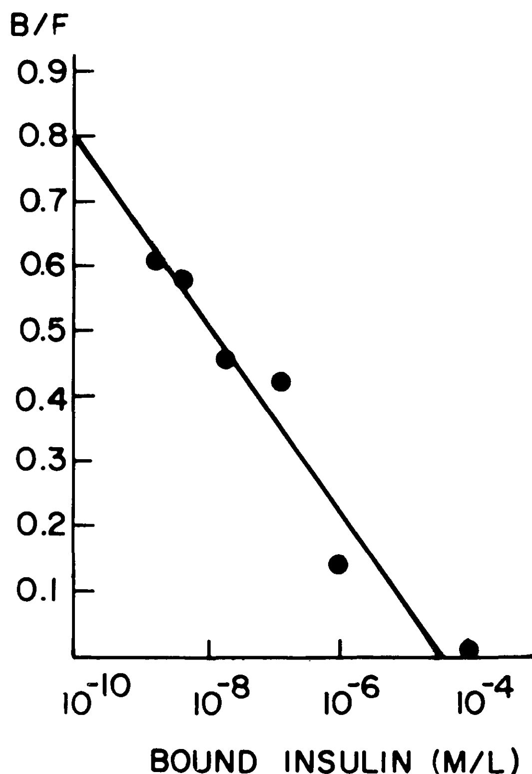

FIGURE 4. Estimation of binding affinity. Competition between ^{125}I-insulin and unlabeled insulin for binding to the monoclonal antibody was used to determine affinity by Scatchard analysis.[24] Bound insulin (B) was precipitated by goat anti-human Fab-*Staphylococcus* and its counts determined. Free insulin (F) was calculated by subtraction of B from the counts per minute of total (T) radiolabeled insulin added. The concentration of bound insulin was computed from the ratio: B (cpm) × C (M/L)/T (cpm), where C is the sum of the concentrations of ^{125}I-Insulin and unlabeled insulin added to each tube.

DISCUSSION

To our knowledge, this is the first report of a human monoclonal antibody to insulin raised from a hybridoma created by fusion with lymphocytes of an IDDM patient. The results of the karyotype determination and the immunoglobulin composition of the culture supernatant (Table 2) indicate that the clone isolated after fusion was a genuine hybrid cell and not a cell resulting from mutation or viral transformation. The frequency of successful fusion was computed to about two hybridomas per 10^7 lymphocytes, a frequency in the range of that reported by others for human-human hybridomas.[18–20]

It is noteworthy that the hybridoma monoclonal antibody to insulin was derived from a patient with undetectable anti-insulin antibodies in his serum. The eight hybridomas obtained from the other patient had no insulin-binding activity (not shown). One might expect that patients with higher titers of antibodies in their serum should have greater numbers of anti-insulin B-lymphocytes. The failure to isolate anti-insulin antibody-producing hybridomas from the patients positive for serum antibodies might have been a coincidence. However, it is conceivable that the B-lymphocytes in these patients that were actively engaged in secreting insulin antibodies were absent from the peripheral circulation or were unsuitable partners for fusion. Be that as it may, patients with low titers of antibodies can be successful candidates for successful generation of hybridomas.

Figure 3 shows that the monoclonal antibody recognized intact porcine insulin, but not isolated A-chain or a mixture of A- and B-chains. These findings indicate that the native conformation was important for antibody binding. This has been shown to be also true for monoclonal anti-insulin antibodies generated in mice.[21] The epitope of insulin recognized by this human antibody was present on human insulin and on insulins of other species including fish, a phenomenon that also has been observed in serum antibodies of IDDM patients.[22] The observation that the antibody recognized a self-determinant defines it as an autoantibody. However, it is not possible to determine whether the antibody was induced by a process of autoimmunization to the patient's own insulin or arose as a consequence of immunization to a cross-reactive determinant present on the exogenous insulin used for treatment.

Estimation of the affinity of the antibody indicated that it had a relatively low affinity for insulin (Figure 4). The clinical significance of covert or low-titer, low-affinity autoimmune anti-insulin antibody production remains to be investigated. A population that should be studied using the hybridoma technique to survey their lymphocytes is that of IDDM patients before insulin treatment. Recently, between 16% and 32% of such individuals were found to have some level of autoimmune anti-insulin antibody in their serum.[23] The observation that the hybridoma antibody inhibited the activity of insulin in lipogenesis (Table 4) suggests that it may be clinically important.

ACKNOWLEDGMENTS

This study was supported by a grant from the Juvenile Diabetes Foundation to I.R.C. Z.E. is the incumbent of the Mar-

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/35/1/68/354470/35-1-68.pdf by guest on 11 September 2024

shall and Renate Ezralov Chair and I.R.C. is the incumbent of the Mauerberger Professorial Chair in Immunology.

REFERENCES

[1] Yalow, R. S., and Berson, S. A.: Immunologic aspects of insulin. Am. J. Med. 1961; 31:882–91.

[2] Culter, L., and Ehrlich, R. B.: Insulin resistance developing in children with IDDM. Diabetes Care 1982; 5:305–10.

[3] Wishner, K. L., and Fisher, L. K.: Insulin allergy in a 19-month-old boy with newly diagnosed diabetes mellitus. Pediatrics 1982; 70:137–38.

[4] Reeves, W. G., Allen, B. R., and Tattersall, R. B.: Insulin-induced lipoatrophy: evidence for an immune pathogenesis. Br. Med. J. 1980; 280:1500–503.

[5] Köhler, G., and Milstein, C.: Continuous cultures secreting antibody of predefined specificity. Nature 1975; 256:495–97.

[6] Corce, C. M., Linnenbach, A., Hall, W., Steplewski, Z., and Koprowski, H.: Production of human hybridomas secreting antibodies to measles virus. Nature 1980; 288:488–89.

[7] Edwards, P. A. W., Smith, C. M., Neville, A. M., and O'Hare, M. J.: A human-human hybridoma system based on a fast-growing mutant of ARH-77 plasma cell leukemia-derived line. Eur. J. Immunol. 1982; 12:641–47.

[8] Littlefield, J. W.: Selection of hybrids from matings of fibroblasts in vitro and their presumed recombinants. Science 1984; 145:709–10.

[9] Ben-Neriah, Y., Wuilmart, C., Lonai, P., and Givol, D.: Preparation and characterization of anti-framework antibodies to the heavy chain variable (Vh) of mouse immunoglobulins. Eur. J. Immunol. 1978; 8:797–801.

[10] Nisonoff, A.: Enzymatic digestion of rabbit gammaglobulin and antibody, and chromatography of digestion products. *In* Methods in Medical Research. Eisen, H. N., Ed. Chicago, Year Book Medical Publishers, 1964:10:134–41.

[11] Fuchs, S., and Sela, M.: Immunoadsorbents. *In* Handbook of Experimental Immunology, 2d edit. Weir, D. M., Ed. Oxford, Blackwell Scientific Publications, 1973; 1:11.1–5.

[12] Hunter, R.: Standardization of the chloramine-T methods of protein iodination. Proc. Soc. Exp. Biol. Med. 1970; 133:989–92.

[13] Hirs, C. H. W.: Performic acid oxidation. *In* Methods in Enzymology, Hirs, C. H. W., Ed. New York, Academic Press, 1967:11:197–99.

[14] Worton, R. G., and Duff, C.: Karyotyping. *In* Methods in Enzymology. Jakoby, W. B. and Paster, I. H., Eds. New York, Academic Press, 1979:58:322–44.

[15] Moody, A. J., Stan, M. A., Stan, M., and Gliemann, J.: A simple free fat cell bioassay for insulin. Horm. Metab. Res. 1974; 6:12–16.

[16] Rodbell, M.: Metabolism of isolated fat cells. I. Effects of hormones on glucose metabolism and lipolysis. J. Biol. Chem. 1964; 239:375–80.

[17] Cuatrecasas, P.: Affinity chromatography and purification of the insulin receptor of liver cell membranes. Proc. Natl. Acad. Sci. USA 1972; 69:1277–81.

[18] Schoenfeld, Y., Hsu-Lin, S. C., Gabriels, J. E., Silberstein, L. E., Furie, B. C., Furie, B., Stollar, B. D., and Schwartz, R. S.: Production of autoantibodies by human-human hybridomas. J. Clin. Invest. 1982; 70:205–208.

[19] Sikora, K., Alderson, T., Phillips, J., and Watson, J. V.: Human hybridomas from malignant gliomas. Lancet 1982; 2:11–14.

[20] Cote, R. J., Morrissey, D. M., Houghton, A. N., Beattie, E. J., Jr., Oettgen, H. F., and Old, L. J.: Generation of human monoclonal antibodies reactive with cellular antigens. Proc. Natl. Acad. Sci. USA 1983; 80:2026–30.

[21] Schroer, J. A., Bender, T., Feldmann, R. J., and Kim, K. J.: Mapping epitopes on the insulin molecule using monoclonal antibodies. Eur. J. Immunol. 1983; 13:693–700.

[22] Karlsson, F., Harrison, L. C., Kahn, C. R., Itin, A., and Roth, J.: Subpopulations of antibodies directed against evolutionarily conserved regions of insulin molecule in insulin-treated patients. Diabetologia 1982; 23:488–93.

[23] Palmer, J. P., Asplin, C. N., Clemons, P., Lyen, K., Tatpati, O., Raghu, P. K., and Paquette, T. L.: Insulin antibodies in insulin-dependent diabetics before insulin treatment. Science 1983; 222:1337–39.

[24] Goldman, J., Baldwin, D., Pugh, W., and Rubenstein, A. H.: Equilibrium binding assay and kinetic characterization of insulin antibodies. Diabetes 1978; 27:653–60.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/35/1/68/354470/35-1-68.pdf by guest on 11 September 2024