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Polyclonal and Monoclonal Antibodies Directed against SK & F 94461, a Specific H_1 Histamine Receptor Ligand

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SUMMARY

SK & F 94461, an aminopentyl analogue of mepyramine, is a recently described H_1 receptor antagonist. At variance with the other available H_1 receptor ligands, SK & F 94461 offers the possibility of coupling to a protein carrier to render the molecule immunogenic. SK & F 94461 coupled to succinylated bovine serum albumin was used as an immunogen to raise polyclonal antibodies in rabbits and BALB/c mice. In parallel, spleen cells from immunized mice were used to produce hybridomas by somatic cell fusion. Thus, six different murine monoclonal antibodies sharing anti-SK & F 94461 specificity were selected for further detailed characterization of their binding properties. Pharmacologic studies of competitive inhibition using a set of 11 histaminergic agents allowed analysis of the fine specificity of anti-SK & F 94461 antibodies. Both polyclonal and monoclonal anti-SK & F 94461 antibodies showed very high affinity for the immunizing molecule (i.e., K_a values for monoclonal antibodies 8 and 12 were, respectively, 3×10^{10} and 1.4×10^{10} M^{-1}). Both types of antibodies bound with high affinity (IC_{50} ranging from 10^{-10} to 10^{-12} M) to mepyramine, which has a chemical structure closely resembling that of SK & F 94461. Moreover, these antibodies displayed clear-cut stereoselectivity inasmuch as they bound the *d*-configuration of chlorpheniramine with significantly higher affinity than the *l*-form. Thus, all six monoclonal antibodies showed IC_{50} values 1 to 6 log units lower for *d*- than for *l*-chlorpheniramine. For some monoclonal antibodies, spectroscopic and fluorescence spectra studies showed that their different binding capacities correlated with their optical properties. Similarly, polyclonal anti-SK & F 94461 antibodies showed a 500-fold lower affinity for *l*- than for *d*-chlorpheniramine. All these results indicate that the polyclonal and the majority of monoclonal anti-SK & F 94461 antibodies recognized with high affinity structural configurations known to be important for the pharmacologic activity of H_1 ligands, namely the presence of the dimethylaminoethyl side chain and, with stereochemical selectivity, the *d*-configuration of chlorpheniramine. These data extend for the first time to an H_1 histamine receptor ligand results reported in other hormone systems.

The precise molecular structure of histamine receptors is still largely unknown. Classification of the various types of histamine receptors (H_1, H_2, and H_3) has mainly been accomplished (1, 2) by careful pharmacological studies using a range of chemical structures having selective agonist or antagonist properties. The problem of detection is further complicated by the fact that radiolabeled ligands permitting sufficiently sensitive and specific characterization of histamine receptors are only available for the H_1 type. Moreover, at present, even for H_1 histamine receptors the reported biochemical data are limited to solubilization procedures (3), molecular weight determination (4), and some studies of receptor chemistry (5).

Based on the encouraging results obtained in several hormonal receptor models, an immunologic approach to the characterization of H_1 histamine receptors was attempted. It has been shown that antihormone (or antiligand) antibodies (Ab1 raised in animal 1) express at or near their binding sites a series of antigenic determinants termed idiotypes that can trigger an immune response when injected into a naive syngeneic animal (animal 2). Thus Ab1 antibodies give rise to anti-idiotypic antibodies (anti-Ab1 or Ab2) some of which, like the initial antigen (i.e., the hormone or the ligand) are able to bind the original receptor. At present, two different but not mutually exclusive hypotheses have been proposed to explain this cross-reactivity. Firstly, anti-idiotypic antibodies would recognize similar antigenic determinants expressed both by the Ab1 binding site and the receptor molecule (6). Alternatively, according to Jerne's network theory (7), anti-idiotypic antibodies (Ab2) express particular idiotypic determinants termed "internal images" that mimic the hormone because they are recognized both by Ab1 antibodies and by the receptor. The present work describes the production of both PoAbs and MoAbs, raised

ABBREVIATIONS: PoAb, polyclonal antibody; MoAb, monoclonal antibody; BSA, bovine serum albumin; ELISA, enzyme-linked immunosorbent assay; CFA, complete Freund's adjuvant; PBS, phosphate-buffered saline; Lys, lysozyme; IEF, isoelectrofocusing.

against SK & F 94461, an aminopentyl analogue of mepyramine. This recently described H_1 receptor antagonist (8) combines the high selectivity of mepyramine and the possibility of being coupled to a protein carrier to be used as an immunogen. The final aim of this study was to delineate the pharmacologic specificity of these various antibodies to assess whether they could represent good candidates for the subsequent production of anti-idiotypic antibodies expressing antireceptor binding capacities.

Materials and Methods

Chemicals

The chemicals used in this study and their sources were as follows: SK & F 94461, bolpyramine, iodobolpyramine, SK & F 47437, and cimetidine from Smith Kline & French (Welwyn, England); triprolidine and *d*- and *l*-chlorpheniramine from Burroughs Wellcome (Research Triangle Park, NC); mianserin from Organon (West Orange, NJ); mepyramine from Specia (Paris, France); doxepin from Merrell Dow (Cincinnati, OH); and brompheniramine racemate from Dexo (Nanterre, France). *N*-Succinimidyl-3-(4-hydroxy-5 [^{125}I]iodophenyl) propionate (Bolton-Hunter reagent; 2000 Ci/mmole) was from Amersham International (Buckinghamshire, England). Histamine and BSA fraction V and Lys were from Sigma Chemical Company (St. Louis, MO).

Preparation of SK & F 94461 Conjugates

SK & F 94461 was coupled to succinylated BSA or Lys by means of carbodiimide as described elsewhere (9). After extensive dialysis, the amount of uncoupled ligand present in the dialysate was determined by competitive inhibition of [^{125}I]iodobolpyramine binding on guinea pig cerebellum membranes. By comparing this value with the initial amount of SK & F 94461 used for the reaction, the number of coupled ligand molecules was deduced. Results showed that a coupling of approximately seven molecules of ligand per molecule of protein was achieved.

Preparation of [^{125}I]Iodobolpyramine

The radiolabeled ligand used in the screening of the antibodies was prepared as previously described (8). Briefly, SK & F 94461 dimaleate, i.e., *N*-(5-aminopentyl)-*N'*- (4 methoxybenzyl)-*N*-methyl-*N'*-2-pyridinyl-1,2-ethanediamine dimaleate, was converted into [^{125}I]iodobolpyramine, i.e., *N*-[5-[3-(4-hydroxy-5-[^{125}I]iodophenyl)propionamido] pentyl]-*N'*-(4 methoxybenzyl)-*N*-methyl-*N'*-2-pyridinyl-1,2-ethanediamine, by *N*-succinimidyl 3-(4-hydroxy, 5-[^{125}I]iodophenyl)propionate based on the method of Bolton and Hunter (10) as follows: SK & F 94461 (10 μg in 10 μl of 0.1 M borate buffer, pH 8.5) was added to the dried ^{125}I-Bolton-Hunter reagent (1 mCi). After 15 min at room temperature, the mixture was spotted onto a thin layer chromatography silica gel plate (60F 254; Merck, Darmstadt, West Germany) and the chromatogram was developed for 10 hr in butanol/acetic acid/water (4:1:1). After being located by autoradiography (Agfa-Gevaert D7), the [^{125}I]iodobolpyramine spot (R_F = 0.33) was scraped from the plate and extracted with 500 μl of ethanol. Solutions were stored at −20° until use.

Cell Lines and Media

The myeloma cell line PAI was kindly provided by Dr. A. D. Strosberg (Institut Pasteur, Paris, France). The cells were cultured at 37° in a humidified atmosphere containing 10% CO_2 in RPMI 1640 medium (GIBCO, Paisley, Scotland) supplemented with 10% fetal calf serum (GIBCO), L-glutamine (1 mM), penicillin (100 IU), and streptomycin (100 mg/ml) (Sigma).

Production of Rabbit and Mouse Anti-SK & F 94461 PoAbs

Four male New Zealand white rabbits (about 400 g) were immunized by footpad and subcutaneous injections of 1 mg of SK & F 94461-BSA (weight of protein) conjugate per rabbit in CFA (Sigma); blood collected before immunization was used as the negative control in the different screening tests of the antibodies obtained. A first booster injection (1 mg of SK & F 94461-BSA in CFA subcutaneously) was performed 1 month after the first injection. Twelve consecutive blood samples were collected once a week after the first booster injection. A second booster injection was performed 13 weeks after the first one and blood was collected once a week for 2 consecutive months. PoAbs were also raised in mice according to the procedure described by Tung (11). Briefly, seven 10- to 12-week-old female BALB/c mice were injected intraperitoneally with 500 μg/mouse of SK & F 94461-BSA (9 parts of CFA for 1 part of antigen solution). Each mouse received 0.2 ml of this emulsion intraperitoneally on day 0, 14, 21, 28, and 35. The majority of mice developed ascitic fluids between day 21 and 35. The ascites were tapped every week for 3–6 months, centrifuged at 1300 × *g* for 20 min at 4° and stored at −20° until used.

Mouse PoAbs were also obtained by conventional immunization of 5- to 6-week-old BALB/c mice. SK & F 94461-BSA (100 μg/mouse) emulsified in CFA was injected at day 0 in the near footpads and subcutaneously in the flanks. Two subcutaneous booster injections (10 μg of SK & F 94461-BSA per mouse) in incomplete Freund's adjuvant were then given at 4 and 8 weeks. Mice were bled at day 10 after the second booster injection.

Somatic Cell Fusions

Spleens from mice immunized according to the conventional procedure were used for fusion experiments. The spleens were excised 3 days after a final intravenous boost with 50 μg of SK & F 94461-BSA. Fusion was performed according to a modification of the procedure previously described by Köhler and Milstein (12). Briefly, 10^8 splenic cells were fused with 10^7 myeloma cells in the presence of 0.85 ml of 50% polyethyleneglycol 1500 (Merk) added to the cell pellet with gentle agitation at 37°, then washed in RPMI 1640 and resuspended in the medium supplemented with 20% fetal calf serum and 1% L-glutamine.

Aliquots of 1 ml (containing 2 × 10^6 cells) were distributed into flat-bottomed 24-well culture plates (Nunclon, Roskilde, Denmark). After 24 hr at 37° under 10% CO_2, 1 ml of fresh medium containing hypoxanthine (0.1 M) aminopterin (0.5 μM), and thymidine (0.1 μM) (HAT medium) was added to the cell cultures. At day 7, 1 ml of the selective medium was removed and replaced with fresh medium. After day 15, the wells were screened visually for cell growth and the culture supernatants were tested for the presence of total immunoglobulins and specific anti-SK & F 94461 antibodies by means of an ELISA.

Subcloning of Positive Hybridomas

Hybridoma cell populations identified by ELISA as secreting anti-SK & F 94461 antibodies were subcloned by limiting dilution (13) (0.5, 1, and 10 cells/well) on feeder layers of DBA/2 mice thymocytes (0.5 × 10^6 cells/ml) in microtiter polystyrene plates (Nunclon). At that time, the selective medium contained only hypoxanthine (0.1 M) and thymidine (0.1 μM) (HT medium). After selection of the wells in which cell growth was detected visually, the culture supernatants were selected by means of ELISA. A second subcloning procedure was performed on positive microwells. The positive cultures were then expanded first into flat-bottomed 24-well culture plates and then into culture flasks (Falcon; Becton-Dickinson Co., Switzerland). Then the hybridomas were amplified into ascitic fluids.

Amplification in Ascitic Form

The amplification was performed in 10- to 12-week-old female BALB/c mice to obtain large concentrations of anti-SK & F 94461 MoAbs. About 2 × 10^6 cells were injected intraperitoneally into mice that had been previously treated with 500 μl of pristane (Coger, Paris, France). Ascites were collected three times a week, centrifuged at 1300 × *g* for 20 min at 4° and stored at −20° until use.

ELISA

An ELISA was used for the detection of immunoglobulins in culture supernatants of hybridoma cell lines as well as for the detection of anti-SK & F 94461 antibodies in polyclonal antisera or ascitic fluids. ELISA microplates (Nunclon) were coated overnight at 4° with the SK & F 94461-lysozyme conjugate diluted in 0.1 M carbonate-bicarbonate coating buffer, pH 9.6 (1 μg in 200 μl/microwell). The plates were then washed extensively with PBS 0.05 M, pH 7.4, containing 0.1% Tween 20 (PBS-Tween) (Sigma) and incubated for 2 hr at room temperature with different dilutions of antibody suspensions in triplicate (diluted in PBS-Tween). The plates were washed again and incubated with the appropriate dilution of horseradish peroxidase-labeled goat antisera specific for mouse (Institut Pasteur Production, Paris, France) or rabbit (Nordic, Tilburg, The Netherlands) immunoglobulins. *o*-Phenylenediamine (Sigma) diluted at 4 mg/ml in phosphate citrate buffer, 0.01 M, pH 5, and activated with 0.01% H_2O_2 was used as the enzyme substrate. The reaction was stopped by 0.1 N H_2SO_4. Absorbance was read at 492 nm (Multiscan; Dynatech SARL, France). Results were expressed for each sample by means of the following index:

$$I = \frac{\text{Mean absorbance of microwells containing the test suspension}}{\text{Mean absorbance of microwells containing the negative control}}$$

The negative control used was, in the case of PoAbs the serum drawn before immunization and, in the case of hybridoma cell supernatants, the supernatant from the nonsecreting myeloma cell line used for fusion.

For isotypic determination of purified mouse MoAbs, rabbit-anti-mouse antibodies directed against the various immunoglobulin subclasses were added to the microwells after reaction of the MoAbs on insolubilized SK & F 94461-Lys. The reaction was revealed using a peroxidase-labeled goat anti-rabbit antisera.

Purification of Anti-SK & F 94461 Antibodies

Rabbit polyclonal antisera and mouse MoAbs and PoAbs were purified as follows: the serum or the ascites were precipitated twice by 50% saturated ammonium sulfate and dialyzed. Then the immunoglobulin fractions were purified by ion-exchange DEAE-Trisacryl chromatography in a 25 mM Tris, 35 mM NaCl buffer, pH 8.8. The purity of the resulting polyclonal or monoclonal immunoglobulins was assessed by electrophoresis on cellulose acetate strips and IEF on polyacrylamide gel. Analytical IEF (pH 3.5 to 10) was performed on a thin layer (0.5 mm) polyacrylamide gel (5%, w/v, acrylamide, 0.15% w/v, bisacrylamide, 0.07%, w/v, ammonium persulfate) containing 0.08%, w/v, ampholytes (LKB, Cambridge, England). The immunoglobulin purified fractions were loaded onto sample applicators (about 3 μl) and focused at a constant current of 50 mA for 1 hr at 4° to a final 2000 V. Different isoelectric point markers were included as reference samples (Pharmacia, Uppsala, Sweden). The gels were fixed in 10% trichloroacetic acid for 1 hr, stained for 30 min with Coomassie blue in acetic acid, methanol, and water (10, 40, and 50%, respectively), and destained in the same solvent.

Binding Characterization of Anti-SK & F 94461 Antibodies

Binding quantification of rabbit polyclonal antisera, mouse polyclonal ascites, and purified mouse MoAbs was performed by means of the Farr immunoprecipitation assay (14). The different dilutions of the test suspensions and the radiolabeled ligand [^{125}I]iodobolpyramine were prepared in a phosphate buffer, $KH_2PO_4/K_2H\ PO_4$, 0.05 M, pH 7.5, containing 0.1% BSA (PBS-BSA), to reduce nonspecific adsorption of [^{125}I]iodobolpyramine on the tubes.

A total of 100 μl of the test suspension, to which 100 μl of the radiolabeled ligand and 100 μl of PBS-BSA were added, was incubated overnight at 4°. The complexes were then precipitated by adding 100 μl of PBS containing 0.5% bovine γ-globulins Cohn Fraction II (Sigma) followed by 500 μl of polyethylene glycol 1500 (Sigma) diluted in the PBS buffer at a 20% final concentration. After a 30-min centrifugation at 1300 × *g* at 4°, the supernatants were removed and the pellet radioactivity was measured in a gamma scintillation counter (Intertechnique, France). The concentration of radiolabeled ligand used in these various assays ranged from 0.020 to 0.025 nM. The blank test contained 100 μl of the radiolabeled ligand and 200 μl of PBS. In competition studies, incubations were performed in the same conditions as described above but in the presence of various concentrations of different histaminergic agents (Fig. 1). The concentrations of ligand giving 50% binding inhibition (IC_{50}) between the antibodies and the radiolabeled marker [^{125}I]iodobolpyramine was deduced from the experimental curves by using an appropriate computer program (15). Binding isotherms of [^{125}I]iodobolpyramine on anti-SK & F 94461 MoAbs 8 and 12 and rabbit PoAbs were performed under the same experimental conditions as described above, using increasing concentrations of radiolabeled ligand (0.005 to 0.3 nM). The affinity constants were then derived by Scatchard analysis (16).

Spectroscopic Studies of Anti-SK & F 94461 MoAbs

Difference UV spectrophotometry. The MoAbs dissolved in PBS (2 ml) at a concentration varying from 1.4 to 2.1 MoAbs μM binding sites were analyzed in a double beam UVIKON P810 spectrophotometer. After baseline storage, 4 μl of a 1 mM PBS solution of *l*-chlorpheniramine or *d*-chlorpheniramine were added, respectively, to the reference cuvette and the sample cuvette. After a 30-sec magnetic stirring, the difference in spectra were recorded using 0.02 absorbance units as full scale. As control for nonspecific binding on the Fc fragment, a MoAb expressing the same isotype (IgG1, termed ANT 7) but differing in its specificity was used.

Difference fluorospectrophotometry. The MoAbs dissolved in

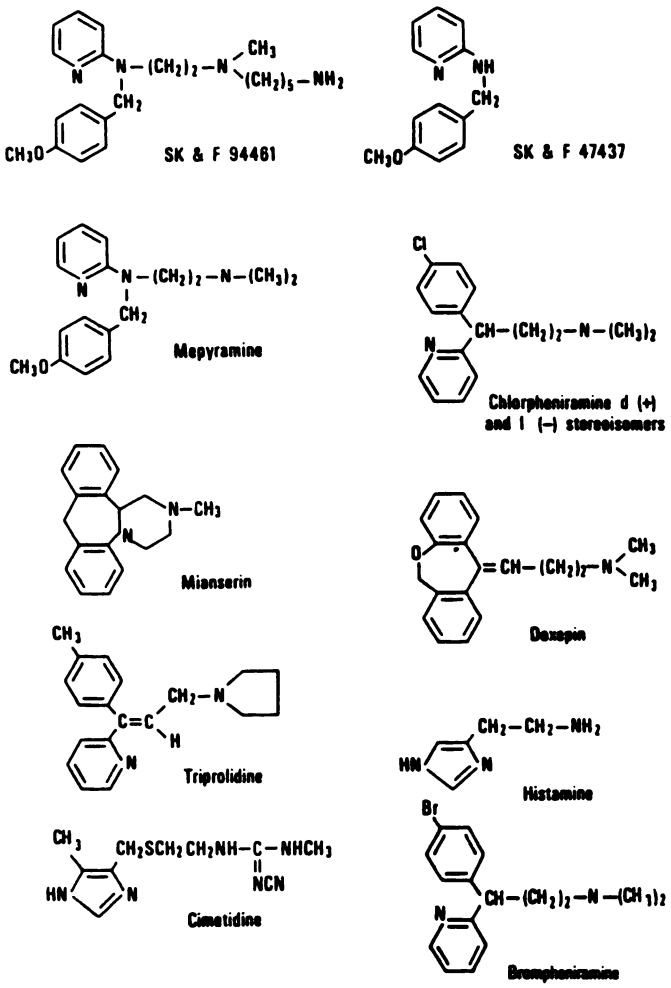

Fig. 1. Chemical structures of histamine and antagonists.

PBS at 0.2 μM binding site concentration (2 ml) were put into a Jobin-Yvon 3D spectrofluorometer. Fluorescence emission was measured from 300 to 380 nm at an excitation wavelength of 285 nm. After determination of the emission spectrum in the absence of ligand, 1 μl of 1 mM *l*-chlorpheniramine solution in PBS was added and the emission spectrum was recorded. Finally, the last emission was recorded on the same sample after addition of the same amount of *d*-chlorpheniramine.

Results

Mouse and Rabbit Polyclonal Antibodies

Production and characterization of mouse PoAbs. High titers of anti-SK & F 94461 PoAbs were observed in the serum of conventionally immunized BALB/c mice (one footpad injection of hapten-carrier conjugate in CFA and subsequent subcutaneous boosts in IFA) after a second booster injection. As for all the other antibodies presented in this study, the sera were tested in parallel by means of ELISA, using insolubilized SK & F 94461-Lys and Farr assay, using the iodinated ligand [^{125}I]iodobolpyramine (mean titers for positive sera in ELISA, 1/1000; mean precipitation in Farr assay, 50%).

A high specific polyclonal response was also observed in the ascitic fluid of BALB/c mice immunized intraperitoneally with the antigen conjugate in CFA according to the procedure described by Tung (11). This protocol, as already described, comprises six injections at different time intervals. Positive ascites could be collected as early as by the third injection (mean titers in ELISA, 1/1000; mean precipitation in Farr assay, 45%).

Production and characterization of rabbit PoAbs. Four rabbits were immunized with the SK & F 94461-BSA conjugate. Two of the four animals (nos. 2 and 4) showed a significant antibody response as early as the seventh day after the first booster injection. In quantitative terms, the response was higher in rabbit 4 than in 2. After the second booster injection all four animals developed high titers of anti-SK & F 94461 PoAbs. The kinetics of this response are described (for rabbits 3 and 4) in Fig. 2.

Although rabbit PoAbs showed positive reactions in both ELISA and Farr assays, there was, as detailed in Fig. 3, a clear gap between the positivity titers recorded by means of these two assays (i.e., higher titers were observed with the Farr assay). Thus, after the first booster injection, as mentioned above, only two of the four rabbits immunized showed a positive reaction in both assays. Conversely rabbits 1 and 3 were positive in ELISA but negative in the Farr assay. These discrepancies may be related to the presence of antibodies that specifically recognize antigenic determinants expressed at the joining region of the coupled ligand. These determinants are absent from the iodinated ligand, thus explaining the differential reactivity observed. Alternatively, the antibody avidity measured in a solid phase assay could be different from the affinity as measured in a liquid phase. Thus, lower affinity antibodies would only be detected by ELISA and not by Farr assay.

Fine specificity of mouse and rabbit PoAbs. Pharmacological studies were performed with a set of histaminergic agents in competitive inhibition experiments, testing the capacity of each given substance to inhibit the binding of [^{125}I] iodobolpyramine to the PoAbs. Both mice and rabbit PoAbs showed a very high affinity for SK & F 94461, i.e., the immunizing hapten (the IC_{50} being $1.6 \pm 0.2 \times 10^{-10}$ M and $0.7 \pm 0.1 \times 10^{-11}$ M, respectively, for mice and rabbit antibodies) (Table 1). Moreover, with mepyramine, which has a chemical structure closely related to that of the immunizing hapten (Fig. 1), they also showed a similar pattern of effective competition (Table 1).

Rabbit PoAbs showed a clear-cut difference in their affinity for the *d*- and *l*-chlorpheniramine stereoisomers. The compe-

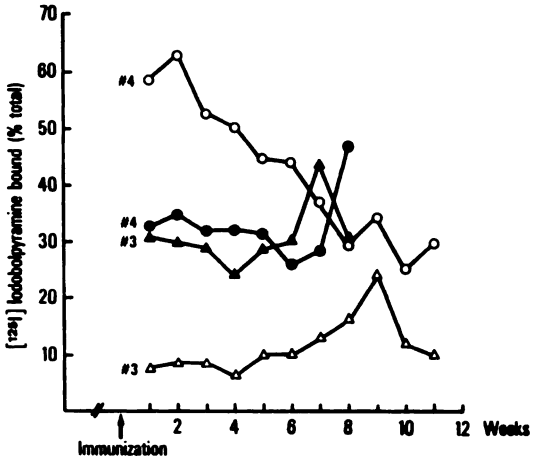

Fig. 2. Kinetics of appearance of anti-SK & F 94461 antibodies in the serum of immunized rabbits (nos. 3 and 4) after the first booster injection (*open symbols*) and the second booster injection (*closed symbols*). Antibodies were detected by means of the Farr assay using [^{125}I]iodobolpyramine. All sera were tested at four different dilutions (1/100, 1/10,000, 1/20,000, and 1/40,000). This figure details the results obtained with 1/40,000 dilution. In rabbit 4 the second booster injection did not induce an immediate increase in antibody serum levels; similar values were found with the serum collected 12 weeks after the first boost (i.e., just before the second) and 1 to 8 weeks after the second boost. Levels began to raise only by the ninth week.

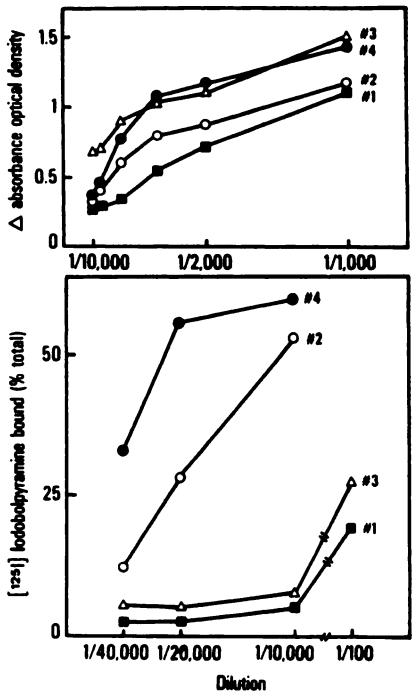

Fig. 3. Correlation between the level of serum anti-SK & F 94461 PoAbs from four immunized rabbits, as assessed by means of ELISA (using insolubilized SK & F 94461-Lys) or Farr assay using [^{125}I]iodobolpyramine. Sera included in this figure were drawn 2 weeks after the first booster injection.

TABLE 1
Binding characteristics of rabbit PoAbs

	[^{125}I]Iodobolpyramine Binding		
	Rabbit PoAbs, IC_{50}[a]	Guinea Pig Cerebellum IC_{50}[b]	Guinea Pig Ileum Contraction, K_a[c]
		nM	
H_1 agonist			
Histamine	196,333 ± 89,666	61,020	
H_1 antagonists			
Mepyramine	0.01	0.59	0.4
Triprolidine	180,000 ± 10,000	0.39	0.1
Doxepin	72,000 ± 1,000	0.21	0.06
Mianserin	327,000 ± 103,000	0.39	2.0
d-Chlorpheniramine	120 ± 8	0.37	0.5
l-Chlorpheniramine	64,000 ± 5,700	63	14
SK & F 94461	<0.007	14.94	32
SK & F 47437	303 ± 77.3	200,000	5250[d]
H_2 antagonist			
Cimetidine	NC[e]	>48,600	446,000

[a] In these experiments each histaminergic competitor has been used at six to eight different concentrations; the concentration of [^{125}I]iodobolpyramine used ranged from 0.020 to 0.025 nM. Values are mean ± standard deviation.
[b] The values reported were deduced from experiments reported in detail in Ref. 8.
[c] K_a is the apparent dissociation constant as defined by inhibition of histamine-stimulated contractions of the guinea pig ileum. Values reported for all ligands but SK & F 47437 are taken from Ref. 8.
[d] M. E. Parsons, personal communication.
[e] NC, no competition.

tition curves recorded with *l*-chlorpheniramine exhibit a 3-log shift to the right, compared with those obtained with the *d*-stereoisomer. It is worth emphasizing at this point that the same stereospecificity, (higher affinity for *d*- than for *l*-chlorpheniramine) as far as the order of recognition of the compounds is concerned, is displayed by the guinea pig H_1 histamine receptor. We were unable to find other H_1-specific *l*- and *d*-isomers that could have been tested in parallel with *l*- and *d*-chlorpheniramine.

Rabbit PoAbs showed a clear-cut shift to the right in effective competition with SK & F 47437 (a partial structure of SK & F 94461), as compared with mepyramine (Table 1). For all the other H_1 antagonists, namely doxepin, triprolidine, and mianserin, as well as for histamine itself, the IC_{50} observed ranged from 0.6 to 3.2 × 10^{-4} M (Table 1). No inhibition was observed with the H_2 receptor antagonist cimetidine.

Mouse Monoclonal Antibodies

Production and characterization. The six MoAbs detailed in this study were recovered from three different somatic cell fusions. Two to four weeks after the fusion, 20 of 120 wells contained stable hybridomas secreting specific antibodies. Ten of these positive wells were submitted to cloning and subcloning procedures. All supernatants were screened by ELISA against the insolubilized SK & F 94461-Lys. The various subclones were amplified in ascites and then tested by both ELISA, against SK & F 94461-Lys, and Farr assay, against [^{125}I]iodobolpyramine. All the ascitic fluids that were positive in ELISA showed positive reactions in the Farr assay. The MoAbs showing the higher responses in the Farr assay were selected for purification and further pharmacologic analysis. Isotypic determination by means of ELISA was performed on purified MoAbs; MoAbs 3 and 7 were IgMk and MoAbs 5, 8, 12, and 13 were IgG_1k immunoglobulins. The monoclonality of the antibodies studied was confirmed by IEF.

Fine specificity of mouse MoAbs. Considering the precise affinity observed for each given agonist and/or antagonist, it is clear that each MoAb exhibited a unique reactivity pattern against the various inhibitors tested. Very high effective competitions were always observed for SK & F 94461 itself, IC_{50} ranging from 0.59 × 10^{-9} M up to 0.90 × 10^{-12} M depending on the MoAb (Table 2). In the case of MoAbs 8 and 12, Scatchard analysis of binding isotherms revealed K_a values of 3 × 10^{10} and 1.42 × 10^{10} M^{-1}, respectively (Fig. 4 describes the case of MoAb 8). Moreover, similar values were recorded for mepyramine (IC_{50} ranging from 0.33 × 10^{-8} to 1.16 × 10^{-12} M) and when analyzing five out of six MoAbs (nos. 3, 5, 8, 12, and 13) for *d*-chlorpheniramine (IC_{50} ranging 1.85 × 10^{-9} to 0.81 × 10^{-11} M) (Fig. 5; Table 2). Interestingly, like PoAbs, all the MoAbs but one (no. 7) made a clear distinction between the two chlorpheniramine stereoisomers; namely, the *l*-configuration of the molecule always showed inhibition curves shifted 4 to 6 orders of magnitude to the right, as compared with the *d*-configuration (Fig. 5; Table 2).

The use of triprolidine and doxepin as competitors allowed a clear-cut distinction among the different MoAbs tested. In competition assays using triprolidine, significantly higher IC_{50} values were observed with MoAbs 5, 7, and 12, as compared with MoAbs 3, 8, and 13 (Table 2). In competition assays using doxepin, only one MoAb showed a high affinity (MoAb 8, IC_{50} = 1.28 × 10^{-8} M), three MoAbs showed intermediate responses (MoAbs 3, 5, and 12), and the two last MoAbs (MoAbs 7 and 13) exhibited 10^{-3} M and 10^{-4} M IC_{50} values. Furthermore, using brompheniramine as a competitor, MoAbs 5, 8, 12, and 13 showed IC_{50} values in the same range as those observed for triprolidine.

None of the MoAbs tested showed any significant recognition of SK & F 47437, which has a chemical structure identical to that of SK & F 94461 but devoid of the entire side chain. Just as for the different PoAbs screened, none of the MoAbs showed any reactivity against either the tetracyclic antidepressant mianserin or the H_2 antagonist cimetidine.

Spectroscopic characterization. Spectroscopy can yield useful information about the aromatic residues involved in protein-ligand interactions. Therefore, UV difference spectra were recorded from 250 to 300 nm between each antibody incubated in the presence of saturating concentrations of *d*-chlorpheniramine (2 μM) or in the presence of the same *l*-chlorpheniramine concentration (which does not bind significantly to the antibody). Two of the three MoAbs (nos. 12 and 13) studied showed a significant decrease in UV absorption between 280 and 260 nm (Fig. 6). The decrease may be related to the combining site of the MoAb because the control MoAb termed ANT 7, (which is an IgG1-like anti-SK & F 94461 MoAb) presenting a nonrelated antigenic specificity, did not show the same pattern in the UV difference spectra (Fig. 6).

Difference fluorescence spectroscopy (Fig. 7) was not significantly different after addition of *d*-chlorpheniramine compared with *l*-chlorpheniramine with the exception of MoAb 12, which showed a positive increase in fluorescence emission between 300 and 320 nm, rising to an 18% increase at 305 nm.

Discussion

PoAbs and MoAbs have been obtained expressing high affinity for SK & F 94461, a recently described specific ligand of the H_1 histamine receptor. The analysis of the fine specificity of all these antibodies using different histaminergic agents (i.e.,

TABLE 2
Binding characteristics of mouse MoAbs

	[^{125}I]Iodobolpyramine, IC_{50}[a] nM					
MoAb	3	5	7	8	12	13
H_1 agonist						
Histamine	NC[b]	NC	NC	NC	NC	NC
H_1 antagonist						
Triprolidine	9.40 ± 2.80	6.90 ± 1,200	14,500 ± 4,066	407.60 ± 30.4	1,900 ± 148	350 ± 56
Brompheniramine	ND[c]	29,800 ± 4,700	ND	620.30 ± 14.90	3,600 ± 817	250 ± 64
Doxepin	350 ± 16	330 ± 13.7	490,000 ± 16,233	12.80 ± 3.28	188 ± 14	51,000 ± 11,300
Mianserin	ND	NC	NC	NC	NC	NC
d-Chlorpheniramine	0.74 ± 0.01	1.85 ± 0.25	913 ± 258	0.008 ± 0.0006	0.78 ± 0.11	0.31 ± 0.08
l-Chlorpheniramine	>10,000	>10,000	>10,000	>10,000	>50,000	>3,000
Mepyramine	ND	ND	0.19 ± 0.03	3.30 ± 1.04	<0.007	<0.001
SK & F 94461	<0.03	<0.03	0.59 ± 0.06	<0.021	<0.0003	<0.0009
SK & F 47437	>440,000	>440,000	363,000 ± 123,000	64,000 ± 12,800	>440,000	>440,000
H_2 antagonist						
Cimetidine	NC	NC	NC	NC	NC	NC

[a] Values are reported as the mean ± standard deviation of the data recovered from five separate experiments.
[b] NC, no displacement observed in the competition assay at 10^{-3} M concentration.
[c] ND, not done.

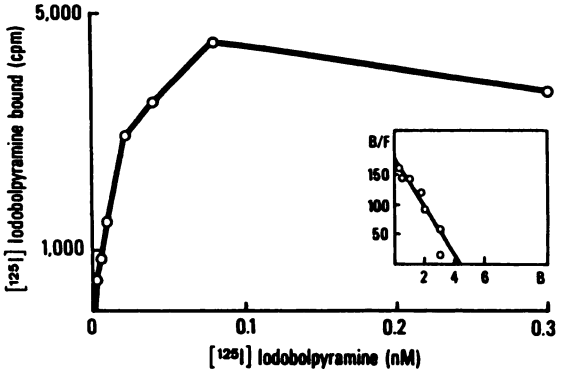

Fig. 4. Binding isotherm of [^{125}I]iodobolpyramine on anti-SK & F 94461 MoAb 8. Purified antibody was incubated overnight at 4° with increasing amounts of [^{125}I]iodobolpyramine and the binding was determined by Farr assay. Scatchard plots were obtained by transformation of the saturable binding curve and showed an association constant of 3×10^{10} M^{-1}.

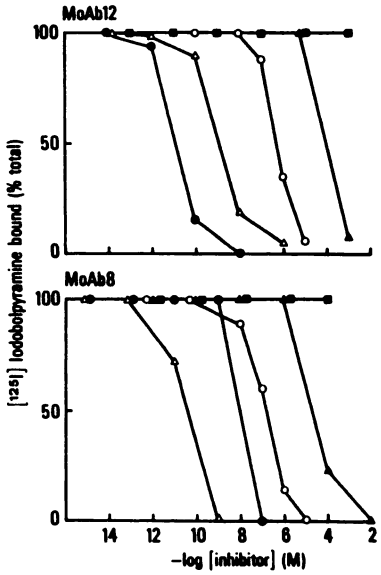

Fig. 5. Competitive inhibition binding curves exhibited by two anti-SK & F 94461 mouse MoAbs (namely, MoAb 12 and MoAb 8) tested for their capacity to bind to [^{125}I]iodobolpyramine in the presence of various histaminergic agents (●, mepyramine; △, *d*-chlorpheniramine; ▲, *l*-chlorpheniramine; ○, triprolidine; and ■, cimetidine). Each competitor has been used at six or seven different concentrations.

several receptor antagonists and histamine itself) revealed unique binding characteristics. Both PoAbs and MoAbs showed a very high affinity for SK & F 94461, with IC_{50} values ranging from 10^{-10} M to 10^{-12} M. Moreover, a nearly similar range of affinities was expressed by both PoAbs and MoAbs against mepyramine, the chemical structure of which closely resembles SK & F 94461. MoAbs appear to recognize the whole of the mepyramine molecule, because they exhibited a very low affinity for the partial structure, SK & F 47437, which lacks the dimethylaminoethyl side chain (IC_{50} values ranging from 10^{-3} to 10^{-4} M). That MoAbs recognize the tertiary amino chain is also suggested by the high affinities they expressed for triprolidine, doxepin, and *d*-chlorpheniramine, which contain such a group. Actually, three of the MoAbs (nos. 3, 5, and 8) expressed high affinities for all three of these histaminergic agents, whereas intermediate recognition patterns were exhibited by MoAbs 7, 12, and 13.

Mianserin, an antidepressant with potent H_1 antagonist properties was not recognized by any of the MoAbs characterized and only slightly by PoAbs ($IC_{50} = 3.2 \times 10^{-4}$ M). The fact that mianserin, although a tertiary amine, lacks the dimethylaminoethyl side chain may explain the absence of recognition. This is further corroborated by the higher affinity displayed by the antibodies for another antidepressant with H_1-antagonist properties, namely, doxepin, which possesses the aminoethyl chain. This therefore seems essential for recognition.

PoAbs also showed preferential reactivity for the whole mepyramine structure. However, the data were less clear-cut than those for MoAbs inasmuch as competition assays using

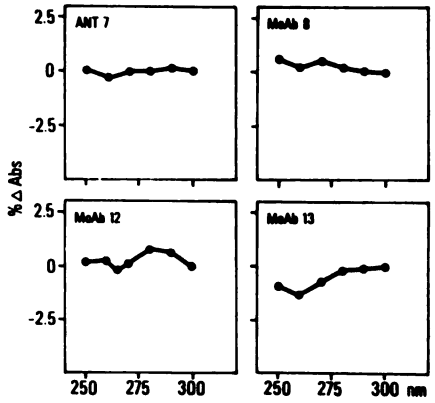

Fig. 6. UV difference absorption spectra of anti-SK & F 94461 MoAbs (1.4 to 2.1 μM binding sites) in the presence of equal amounts (2 μM) of *l*- and *d*-chlorpheniramine diluted in PBS. ANT 7 is the control MoAb. Results are the mean of three consecutive absorption spectra and are expressed as percentage of the absorption change.

SK & F 47437 revealed a mean IC_{50} value of 3×10^{-7} M. Similarly, although they showed significant affinity for *d*-chlorpheniramine ($IC_{50} = 1.2 \times 10^{-7}$ M), doxepin and triprolidine were less readily recognized ($IC_{50} = 7.2 \times 10^{-5}$ and 1.8×10^{-4} M, respectively). At this point, it is interesting to recall that PoAbs represent a mixture of antibody families expressing an heterogeneous specificity pattern that, in the present setting, seems to be well characterized by the analysis of the individual MoAb reactivities.

Of special interest is the finding of stereoselectivity for *d*- and *l*-chlorpheniramine that was evidenced for both PoAbs and the MoAbs, indicating that the antibodies bind the ligands in a stereochemically dependent manner. A shift of the IC_{50} values by 2 to 6 log units to the right was recorded for the six MoAbs analyzed. PoAbs showed a 500-fold lower affinity for *l*- than for *d*-chlorpheniramine. Although the induction of isomer-specific antibodies by an immunogen lacking a geometric or chiral center is not the rule, some examples were recently reported. A MoAb raised against the tricyclic antidepressant nortriptyline could distinguish between the *E*- and the *Z*-forms of the analogue molecule doxepin, in which a geometrical center is introduced by replacing a CH_2 radical by a O atom in the central heptacycle (17). PoAbs raised against a racemic mixture of a nicardipine analogue were able to recognize the (+)-form of nicardipine with a eudismic ratio of 38 (18). In both cases, as well as in the case of the antibodies described here, it can be assumed that the genetic and/or conformational restrictions governing the structure of the antibody combining site favor recognition of one of the isometric configurations potentially present in the asymmetric molecule or the racemic mixture. The presented results are consistent with a three-dimensional interaction involving at least three main sites, *viz.*, the two aryl rings and the amino groups. Because the chiral center for chlorpheniramine is at the carbon atom bearing the two aryl groups, it is evident that the ring systems are being recognized and play an important role in the observed affinity. The high stereospecificity of all antibodies for the chlorpheniramine enantiomers suggests that the antibody sites interacting with the pyridinyl groups are very shallow and do not allow combination with the bulkier chlorophenyl ring. On the other hand, the low affinity for the brompheniramine racemate may indicate that the antibody site interacting with the chlorophenyl group is unable to recognize the bromophenyl group, which is

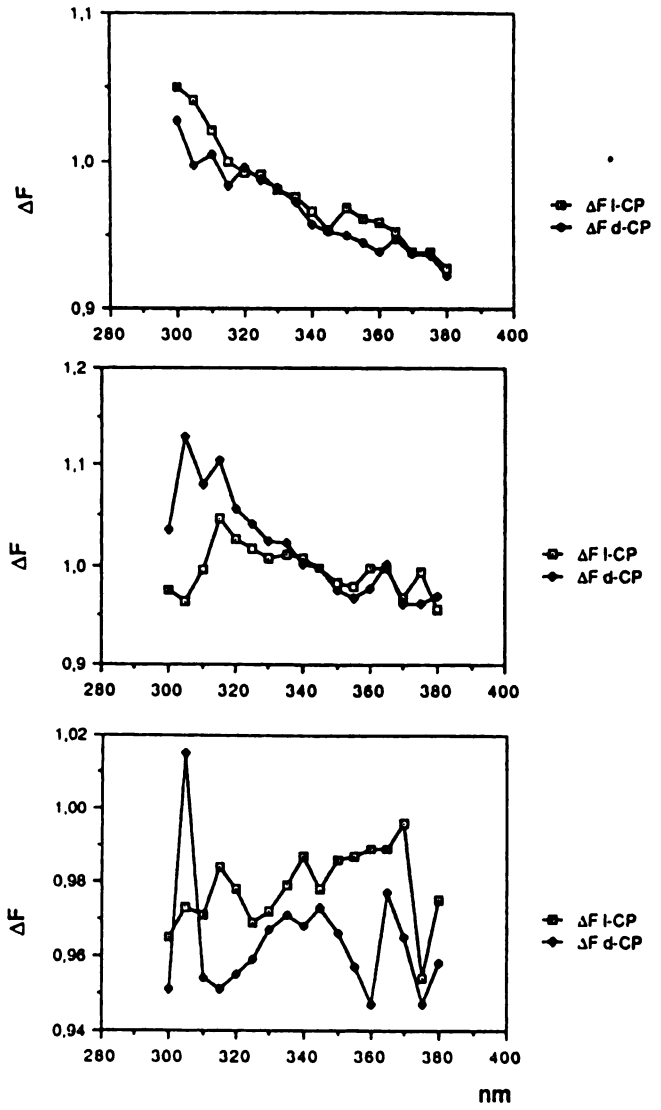

Fig. 7. Difference fluorescence emission spectra of anti-SK & F 94461 MoAbs (0.2 μM binding sites) in the presence of *l*-chlorpheniramine (□) and *d*-chlorpheniramine (■) at 0.5 μM. Δ *F* is the ratio of fluorescence in the presence of ligand to that in the absence of ligand. From *upper* to *lower graph*, difference spectra for MoAb 8, MoAb 12, and MoAb 13 are shown.

bigger. Thus, for *d*-chlorpheniramine both rings bind, whereas for the *l*-form it is probable that only one ring binds and the other is held away from the binding site. The possibility of hydrophobic interactions with the antibody combining site is also suggested by their spectroscopic properties. In one case (MoAb 12) the probable involvement of a tryptophan residue in the interaction with the hapten was suggested by shifts in the UV and fluorescence spectra upon ligand binding. MoAb 13 showed a similar shift in the UV spectrum without concomitant change in the fluorescence spectrum; MoAb 8 did not exhibit significant changes in spectra. These results confirm that the differences in binding properties observed with the MoAbs tested are also reflected in their optical properties.

No H_1 agonists except histamine were tested, as their affinity for the H_1 receptor is low, as is that of histamine, and thus they would not have been useful in defining the orders of binding potencies of the antibodies as compared with the receptor.

Histamine was not recognized by any of the MoAbs tested, and a 1.9×10^{-4} M IC_{50} value was found when analyzing PoAbs.

It is worth stressing that cimetidine, an H_2 receptor antagonist, displayed negligible potency in all the competition assays performed. All these results indicate that, although the precise pharmacology of anti-SK & F 94461 PoAbs and the majority of the MoAbs here described does not strictly correlate with that displayed by the guinea pig H_1 receptor, the antibodies recognize with high affinity structural configurations known to be important for pharmacologic activity, namely the presence of the dimethylaminoethyl side chain and, with stereochemical selectivity, the *d*-configuration of chlorpheniramine. It is worth stressing that, like the presented antibodies, H_1 receptors recognize with low affinity SK & F 47437, the chemical structure of which is identical to that of mepyramine or SK & F 94461 without the aliphatic side chains. In addition, it is important to note that although the antibodies were raised against SK & F 94461, they recognize (except for MoAb 8) mepyramine with nearly equal affinity. Thus, the spacer arm coupled through —NH— present in the hapten carrier immunogen does not contribute to recognition. These data extend for the first time to an H_1 histamine receptor ligand the results that have been reported for β-adrenergic receptor ligands (19–21), the chemoattractant peptide specific for neutrophils (22), opiates (23–24), substance P (25), and recently the D-2 dopaminergic receptor antagonist haloperidol (26).

Several uses may be envisioned for this sort of antiligand antibody. Of course, based on the very encouraging results obtained in all published receptor models, one major objective is to obtain by means of anti-idiotypic immunization either PoAbs or MoAbs directed against the active site of the antiligand immunoglobulins that would display antireceptor activity. By means of this method, antibodies recognizing the insulin (27), thyrotropin (28), acetylcholine (29), glucocorticoids (30), β-adrenergic (31–33), opiate (24, 34), substance P (25), and adenosine receptors (35) have been reported. Interestingly, these antibodies were not only useful for characterizing these receptors but in many cases were also known to possess important functional capacities (36). The question then arises as to whether the aim should be to seek the antibody that recognizes mepyramine or another ligand with the greatest possible affinity (and presumably therefore, specificity) or rather to seek the antibody that recognizes a range of antihistamine structures. The data available from the literature do not provide a basis for drawing firm conclusions because each model presents its unique characteristics. Actually, successful anti-idiotypic immunization giving rise to antibodies displaying antireceptor specificity was carried out using antiligand antibodies fulfilling either one or the other of the two above-mentioned criteria. Thus, anti-idiotypic antibodies showing β-adrenergic receptor specificity have been produced by injecting antialprenolol antibodies recognizing features common to a broad range of β-antagonists and in some cases even agonists. In this particular model, the rank order of recognition of β-adrenergic ligands by the antibodies as compared with the receptor was considered the best criterion even if the precise pharmacology (i.e., absolute affinities of antibodies as compared with the receptor for the various ligands) did not correlate.

In the case of opiate receptors the situation is completely different. Antireceptor anti-idiotypic PoAbs were also obtained by means of immunization with either antimorphine PoAbs or MoAbs (24, 34). However, unlike the opiate receptor, antimorphine PoAbs bound agonists with higher affinity than antagonists (24). Similarly, the antimorphine MoAb used was very selective for the morphine skeleton and no apparent relationship was observed between the binding affinities of this antibody and the rank order of binding potency displayed by the receptor (23). Considering these various reports as a whole, both anti-SK & F 94461 PoAbs and MoAbs exhibiting different recognition patterns have been used for further immunization.

Another possible use for these antiligand antibodies would be the preparation of affinity columns for receptor purification in the case in which the antibodies still recognize the hapten once it is bound to the receptor.

Finally, considering the numerous major functions mediated by histamine in different tissues, it would be of interest to determine whether antihistamine receptor anti-idiotypic antibodies could be detected in some pathologic conditions. In this particular context antiligand antibodies could represent valuable probes.

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References

1. Ganellin, C. R., and M. E. Persons, eds. *Pharmacology of Histamine Receptors* John Wright, Bristol, United Kingdom (1982).
2. Arrang, J. M., M. Garbarg, and J. C. Schwartz. Auto-inhibition of brain histamine release mediated by a novel class H_3 of histamine receptor. *Nature (Lond.)* **302**:832–837 (1983).
3. Warlow, R. S., and C. C. A. Bernard. Solubilization and characterization of moderate and high affinity histamine binding sites on human blood mononuclear cells. *Mol. Immunol.* **24**:27–37 (1987).
4. Takayoshi, K., K. Nobuo, and T. Chikato. Molecular size of histamine H_1-receptor determined by target size analysis. *Biochem. Biophys. Res. Commun.* **129**:639–644 (1985).
5. Garbarg, M., E. Yeramian, M. Korner, and J. C. Schwartz. Biochemical studies of cerebral H_1 receptors, in *Frontiers in Histamine Research* (C. R. Ganellin and J. C. Schwartz, eds.), Vol. 51. Pergamon Press, London, 19–26 (1985).
6. Strosberg, A. D., J. G. Guillet, S. Chamat, and J. Hoebeke. Recognition of physiological receptors by anti-idiotypic antibodies: molecular mimicry of the ligand or cross reactivity? *Curr. Top. Microbiol. Immunol.* **119**:91–110 (1985).
7. Jerne, N. K. Idiotypic networks and other preconceived ideas. *Immunol. Rev.* **79**:5–24 (1984).
8. Korner, M., M. L. Bouthenet, C. R. Ganellin, M. Garbarg, C. Gros, R. J. Ife, N. Sales, and J. C. Schwartz. [^{125}I]Iodobolpyramine, a highly sensitive probe for histamine H_1-receptors in guinea-pig brain. *Eur. J. Pharmacol.* **120**:151–160 (1985).
9. Kamel, R. S., J. Landon, and D. S. Smith. Novel ^{125}I-labeled nortriptyline derivatives and their use in liquid-phase or magnetizable solid-phase second-antibody radioimmunoassays. *Clin. Chem.* **25**:1997–2002 (1979).
10. Bolton, A. E., and W. M. Hunter. The labeling of proteins to high specific radioactivities by conjugation to a ^{125}I-containing acylating agent. *Biochem. J.* **133**:529–535 (1973).
11. Tung, A. S. Production of large amounts of antibodies, nonspecific immunoglobulins, and other serum proteins in ascitic fluids of individual mice and guinea pigs. *Methods Enzymol.* **93**:12–23 (1983).
12. Köhler, G., and C. Milstein. Continuous cultures of fused cells secreting antibodies of predefined specificity. *Nature (Lond.)* **256**:495–498 (1975).
13. Oi, V. T., and L. A. Herzenberg. Immunoglobulin-producing hybrid cell lines. in *Methods in Cellular Immunology* (R. B. Mishell and S. M. Shiji Freeman, eds.). San Francisco. 351–372 (1980).
14. Farr, R. S. A quantitative immunochemical measure of the primary interaction between *I-BSA and antibody. *J. Infect. Dis.* **103**:239–245 (1958).
15. Minneman, K. P., L. R. Hegstrand, and P. B. Molinoff. Simultaneous determination of *beta*-1 and *beta*-2 adrenergic receptors in tissues containing both receptor subtypes. *Mol. Pharmacol.* **16**:34–46 (1979).
16. Scatchard, G. The attractions of proteins for small molecules and ions. *Ann. N. Y. Acad. Sci.* **51**:660–672 (1949).
17. Marullo, S., J. Hoebeke, J. G. Guillet, and A. D. Strosberg. Structural analysis of the epitope recognized by a monoclonal antibody directed against tricyclic antidepressants. *J. Immunol.* **135**:471–477 (1985).
18. Thayer, S. A., D. H. Pham, C. M. Schultz, G. Minaskanian, and A. S.

Fairhurst. An antibody to dihydropyridine calcium entry blockers. *Biochem. Pharmacol.* **35**:4479–4485 (1986).
19. Hoebeke, J., G. Vauquelin, and A. D. Strosberg. The production and characterization of antibodies against β-adrenergic antagonists. *Biochem. Pharmacol.* **27**:1527–1532 (1977).
20. Chamat, S., J. Hoebeke, and A. D. Strosberg. Monoclonal antibodies specific for β-adrenergic ligands. *J. Immunol.* **133**:1547–1552 (1984).
21. Sawutz, D. G., D. Sylvestre, and C. J. Homcy. Characterization of monoclonal antibodies to the β-adrenergic antagonist alprenolol as models of the receptor binding site. *J. Immunol.* **135**:2713–2718 (1985).
22. Marasco, W. A., and F. L. Becker. Anti-idiotype as antibody against the formyl peptide chemotaxis receptor of the neutrophil. *J. Immunol.* **128**:963–968 (1982).
23. Glasel, J. A., M. William, B. F. Venn, and R. F. Venn. Properties of murine anti-morphine antibodies. *Mol. Immunol.* **20**:1419–1422 (1983).
24. David, S. N. G., and G. E. Isom. Binding of antimorphine anti-idiotypic antibodies to opiate receptors. *Eur. J. Pharmacol.* **102**:187–190 (1984)
25. Couraud, J. Y., E. Escher, D. Regoli, V. Imhoff, B. Rossignol, and P. Pradelles. Anti-substance P anti-idiotypic antibodies. *J. Biol. Chem.* **260**:9461–9469 (1985).
26. Sherman, M. A., D. Scott Linthicum, and M. B. Bolger. Haloperidol binding to monoclonal antibodies: conformational analysis and relationships to D-2 receptor binding. *Mol. Pharmacol.* **29**:589–598 (1986).
27. Sege, K., and P. A. Peterson. Use of anti-idiotypic antibodies as cell-surface receptor probes. *Proc. Natl. Acad. Sci. USA* **75**:2443–2447 (1978).
28. Briones-Urbina, R., N. N. Islam. J. Ivanyi, and N. R. Farid. Use of anti-idiotypic antibodies as probes for the interaction of TSH subunits with its receptor. *J. Cell. Biochem.* **34**:151–182 (1987).
29. Wassermann, N. H., A. S. Penn, P. I. Freimuth, N. Treptow, S. Wentzel, W. L. Cleveland, and B. F. Erlanger. Anti-idiotypic route to anti-acetylcholine receptor antibodies and experimental myasthenia gravis. *Proc. Natl. Acad. Sci. USA* **79**:4810–4814 (1982).
30. Cayanis, E., R. Rajagopalan, W. L. Cleveland, I. S. Edelman, and B. F. Erlanger. Generation of an auto-anti-idiotypic antibody that binds to glucocorticoid receptor. *J. Biol. Chem.* **261**:5094–5103 (1985).
31. Homcy, C. J., S. G. Rockson, and E. Haber. An antiidiotypic antibody that recognizes the β-adrenergic receptor. *J. Clin. Invest.* **69**:1147–1154 (1982).
32. Schreiber, A. B., P. O. Couraud, C. Andre, B. Vray, and A. D. Strosberg. Anti-alprenolol anti-idiotypic antibodies bind to β-adrenergic receptors and modulate catecholamine-sensitive adenylate cyclase. *Proc. Natl. Acad. Sci. USA* **77**:7385–7389 (1980).
33. Guillet, J. G., S. V. Kaveri, O. Durieu, C. Delavier, J. Hoebeke, and A. D. Strosberg. β-Adrenergic agonist activity of a monoclonal anti-idiotypic antibody. *Proc. Natl. Acad. Sci. USA* **82**:1781–1784 (1985).
34. Glasel, J. A., and E. Myers. Rabbit anti-idiotypic antibodies raised against monoclonal anti-morphine IgG block μ opiate receptor sites. *Life Sci.* **36**:2523–2529 (1985).
35. Ku, H. H., W. L. Cleveland, and B. F. Erlanger. Monoclonal antibodies to adenosine receptor by an auto-anti-idiotypic approach. *J. Immunol.* **139**:2376–2384 (1987).
36. Strosberg, A. D. Anti-idiotype and anti-hormone receptor antibodies. *Springer Semin. Immunopathol.* **6**:67–78 (1983).

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