Monoclonal antibodies against E- and F-type prostaglandins

High specificity and sensitivity in conventional radioimmunoassays

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Polyclonal antisera against prostaglandins (PGs) are widely used for the assessment of the biological role of these mediators, but even the most specific contain antibodies against the major metabolites and degradation products of the haptens employed. To overcome this inherent problem we produced monoclonal antibodies (mAs) against PGE_2, $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ using the somatic cell hybridization technique. The mAs against 6-keto-$PGF_{1\alpha}$ and $PGF_{2\alpha}$ proved to be highly specific, but allowed only for moderate detection limits (1–2 ng) in conventional fluid phase radioimmunoassays (RIAs). One of the mAs against PGE_2 permitted a 100-fold improvement in the detection limit while being almost devoid of cross-reactivity with metabolites and other structurally related PGs. These results show that highly specific mAs against PGs can be produced to improve the available RIA technique for PG quantification.

Cell hybridization *Prostaglandin E_2* *Prostaglandin $F_{2\alpha}$* *6-Keto-$PGF_{1\alpha}$* *Radioimmunoassay*

1. INTRODUCTION

Prostaglandin (PG) E_2, a major eicosanoid in man, is involved in a variety of physiological and pathological events [1]. Multiple attempts have hence been made to raise antibodies of sufficient specificity to qualify for direct radioimmunoassay (RIA) of E-series PGs in biological samples [2–13]. However, the production of such antibodies (Abs) proved difficult, probably due to the chemical and metabolic instability of the hapten. Dehydration at the 11-hydroxy position resulting in the formation of A- and B-series PGs may occur during the coupling reaction of the hapten to the immunogen as well as by plasma isomerase enzymes [14,15]. The resulting polyclonal antibodies hence often recognize PGE_2, PGA_2 and PGB_2 as well as other structurally related PGs to an extent which excludes them from being used in the specific determination of PGE_2. Some research groups, either by serendipity and specific skills [13,15,16] or due to a specific strategy such as using stable PGE_2 analogues as haptens [17], were able to overcome this problem partially. An alternative approach would be the production of monoclonal antibodies (mAs) as suggested [18] using the cell hybridization technique [19]. A suitable mA against PGE_2 should be devoid of major cross-reactions against PGA_2, PGB_2, other metabolites of PGE_2 and related PGs. Moreover, such mAs can be produced in almost unlimited quantities of a uniform standard in vitro, once a satisfying hybridoma cell line has been produced.

Previously we reported that the production of such cell lines is possible [20]. Now we describe an mA against PGE_2 which compares favourably with polyclonal Abs in that it allows for the development of highly sensitive and specific RIAs.

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2. MATERIALS AND METHODS

2.1. *Materials*

All prostaglandins were generous gifts from Upjohn Co. (Kalamazoo, USA) or bought from Sigma Chemie (Taufkirchen, FRG). [5,6,8,11,12,14,15(n)-^{3}H]PGE_2 (spec. act. 160 Ci/mmol) was purchased from New England Nuclear (Dreieich, FRG). [5,6,8,9,11,12,14,15(n)-^{3}H]$PGF_{2\alpha}$ (spec. act. 180 Ci/mmol), 6-keto[5,8,9,11,12,14,15(n)-^{3}H]$PGF_{1\alpha}$ (spec. act. 150 Ci/mmol) and [5,6,8,11,12,14,15(n)-^{3}H]PGA_2 (spec. act. 160 Ci/mmol) were bought from Amersham Buchler (Braunschweig, FRG). [^{3}H]PGB_2 was prepared according to the method of Zusman [21] from [^{3}H]PGE_2.

2.2. *Methods*

2.2.1. Coupling

The haptens (PGE_2, 6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$) were covalently bound to bovine serum albumin (BSA) with 1-ethyl-3-(3-methylaminopropyl)carbodiimide-HCl (both from Sigma, Taufkirchen) as coupling reagent according to the method of Dray et al. [18].

2.2.2. Immunization

About 8-week-old Balb/c mice (♀ ♀) were first immunized with 20 μg PG-BSA conjugate, emulsified with an equal volume of complete Freund's adjuvant (Difco, Detroit) and injected s.c. in the neck region and i.p. The second injection was given 3 days later in the same way, with incomplete Freund's adjuvant. Booster injections were given every 7 days i.p. with 20 μg antigen. Specific [^{3}H]PG binding of plasma was monitored between the booster injections.

2.2.3. Fusion and selection of clones

Three days after the last booster injection (4 weeks after the first) mice with high-titre plasma were bled and their spleens removed. Fusion of the spleen cells with an aminopterin-sensitive myeloma cell line (X63 Ag8.653, gift from G. Köhler, MPI, Freiburg) was performed using a solution of 42% polyethylene glycol (PEG) 4000 (Merck, Darmstadt) with 15% dimethyl sulfoxide (DMSO) by the method of Köhler and Milstein [19]. Resulting cell suspensions were fed with the selective medium containing hypoxanthine, aminopterin and thymidine. The hybridoma culture supernatants were tested for antibody production by determining specific [^{3}H]PG binding of the corresponding hapten. Selected hybridoma cells were cloned twice by limiting dilution on feeder layers of mouse peritoneal macrophages.

2.2.4. Production of mAs

Clones were expanded in Dulbecco's modified Eagle medium (DMEM) without phenol red (Biochrom, Berlin), completed with 1 mM sodium pyruvate, 2 mM L-glutamine, 0.05 mM 2-mercaptoethanol (Sigma, Taufkirchen) and 20% foetal calf serum (FCS) (all Gibco, Karlsruhe). The culture medium was collected every 3–4 days, concentrated and lyophilized.

2.2.5. Characterization

Sensitivity of RIAs and cross-reactions were determined by binding inhibition assays [22]. The percentage of relative cross-reaction was calculated as

$$\frac{\text{ng homologous inhibitor at 50\% binding of radiolabel}}{\text{ng cross-reactive inhibitor at 50\% binding of radiolabel}} \times 100$$

The sensitivity of RIAs was calculated as the quantity of homologous inhibitor at 90% binding of the radiolabel (10% inhibition of binding as compared to buffer controls). The subclasses of the monoclonal antibodies were identified by an enzyme-linked immunosorbent assay (ELISA), using class-specific rabbit-anti-mouse IgG and goat-anti-mouse IgG (2a,κ,λ) (Nordic Immunol. Lab., Tilburg, The Netherlands). Rabbit-anti-mouse IgG peroxidase and goat-anti-mouse IgG peroxidase were purchased from Dakopatts (Copenhagen).

3. RESULTS

The results are given in table 1 and fig.1. The mAs against $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ showed a high degree of specificity displaying only about 1% cross-reactivity with the structurally related PGE_2, PGE_1 and $PGF_{1\alpha}$ (table 1). These mAs, however, allowed only for the measurement of fairly high concentrations of 6-keto-$PGF_{1\alpha}$ or $PGF_{2\alpha}$ (> 1 ng) when used in the conventional RIA in solution employed by us routinely [23]. In contrast, our first mA against PGE_2 (E_2R_1) allowed for the detection of much smaller amounts of PGE_2

Table 1

Characteristics of monoclonal antibodies against E- and F-type prostaglandins

Prostaglandins	Cross reaction (%)			
	mA-PGE_2 (E_2R_2)	mA-PGE_2 (E_2R_1)	mA-6k-$PGF_{1\alpha}$	mA-$PGF_{2\alpha}$
PGE_2	100	100	1.83	1.32
PGE_1	5.54 (12)	18.5	1.37	2.42
PGA_2	0.32 (5)	n.t.	n.t.	n.t.
PGB_2	0.79	n.t.	n.t.	n.t.
PGD_2	0.12	<1.3	<1	<1
$PGF_{2\alpha}$	0.17	21.6	0.67	100
$PGF_{1\alpha}$	0.33	n.t.	1.83	2.05
TXB_2	<0.05	<1.3	<1	<1
TXB_1	n.t.	n.t.	<1	<1
15-Keto-PGE_2	1.32 (2)	n.t.	n.t.	n.t.
15-Keto-13,14-dihydro-PGE_2	0.97	n.t.	n.t.	n.t.
11-Deoxy-15-keto-13,14-dihydro-11,16-cyclo-PGE_2	<0.05	n.t.	n.t.	n.t.
15-Keto-13,14-dihydro-$PGF_{2\alpha}$	<0.05	n.t.	n.t.	<1
15-Keto-$PGF_{2\alpha}$	<0.05	n.t.	n.t.	<1
6-Keto-$PGF_{1\alpha}$	0.07	100	100	n.t.
6,15-Diketo-$PGF_{1\alpha}$	0.07	n.t.	<1	n.t.
6,15-Diketo-13,14-dihydro-$PGF_{1\alpha}$	0.06	n.t.	<1	n.t.
Arachidonic acid	<0.05	n.t.	n.t.	n.t.
Sensitivity (ng)	0.03 (0.01)	0.22	1.1	2.0
Immunoglobulin subclass	IgG1, $\varkappa$-l.-chains	IgG1, $\varkappa$-l.-chains	IgG1, $\varkappa$-l.-chains	IgG1, $\varkappa$-l.-chains

Values in parentheses give characteristics of a specific antiserum against PGE_2 (32-7) used for comparison [11,22]. n.t., not tested

(>200 pg) but its cross-reaction with 6-keto-$PGF_{1\alpha}$ (100%), PGE_1 and $PGF_{2\alpha}$ was considerable. The second mA against PGE_2 (E_2R_2) proved to be very specific, displaying some cross-reactivity only with PGE_1 (~5%), and 15-keto-PGE_2 (1%), but not with, e.g., 6-keto-$PGF_{1\alpha}$ or a great variety of other PGs. It compared very well in that respect with a widely used antiserum (table 1). In addition, this mA if employed in the conventional fluid phase RIA allowed for the detection of small amounts of PGE_2 (30 pg). In further experiments (fig.1) it was shown that this mA was devoid of the ability to bind [^{3}H]PGA_2 and [^{3}H]PGB_2, which is usually displayed by antisera against PGE_2. As shown in fig.1, even at high concentrations the mA-PGE_2 does not bind PGA_2 or PGB_2 while the antiserum does (for details see [11,24]). The immunoglobulin subclass of all mAs was defined as IgG1 with $\varkappa$-light chains.

4. DISCUSSION

Our results show that it is possible to raise hybridoma clones which produce monoclonal antibodies against PGs in vitro. Some of these mAs can be used in conventional RIAs, allowing for the direct and sensitive detection of biologically relevant PGs. For example, our mA-PGE_2 (E_2R_2) is

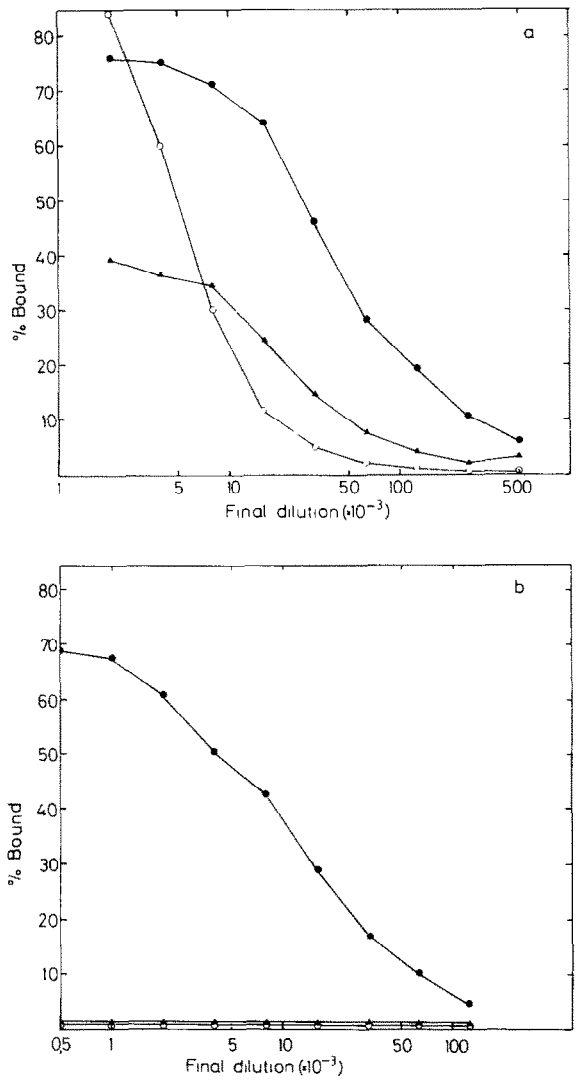

Fig.1. (a,b) Binding of [^{3}H]PGE_2 (●), [^{3}H]PGA_2 (○) and [^{3}H]PGB_2 (▲) at equal specific activity by dilutions of (a) specific polyclonal antiserum (32-7), (b) mA-PGE_2 (E_2R_2) culture supernatant.

now routinely used in a standard RIA assessing the prostaglandin release from macrophages yielding almost identical results as compared to a well defined specific antiserum against PGE_2 ([11,24] and table 1). The mA-6-keto-$PGF_{1\alpha}$ and mA-$PGF_{2\alpha}$ are not of comparable quality in conventional fluid phase assays due to relatively high detection limits. They have, however, proven useful when employed in an immunosorbent assay on plastic surface to be reported elsewhere.

The mA concentration in hybridoma supernatants is high enough for the direct use of these supernatants in the assay procedure. For example, the dilutions given in the figure are direct dilutions from pooled hybridoma supernatants harvested after approx. 3 days (cell count at that time ~5 × 10^6/ml). In other words, it is not necessary to initiate the production of mAs in the peritoneal cavity of Balb/c mice for sufficient mA concentrations. Furthermore, our data prove that it is possible to circumvent problems inherent to conventionally produced polyclonal antisera. The mA-$PGF_{2\alpha}$, mA-6-keto-$PGF_{1\alpha}$ and the mA-PGE_2 (E_2R_2) exert a high degree of specificity, i.e., these antibodies can discriminate well between minor structural differences in the side chains of PGs and in the ring structure. The mA-PGE_2 (E_2R_2), for example, is able to detect minor changes in the side-chain configuration as present in PGE_1 or in 15-keto-PGE_2 although with some cross-reactivity, but there is no detectable cross-reactivity with $PGF_{2\alpha}$, PGA_2 or PGB_2 which differ in the substituent configuration of the cyclopentane ring. The small (5%) cross-reactivity with PGE_1 is less than with even the best anti-PGE_2 antisera. Furthermore, it is of little relevance under most experimental conditions due to the low production of PGE_1 by most mammalian cells [25]. Moreover, the inherent problem of PGE_2-antisera, namely, that they contain antibodies against PGA_2 and PGB_2 [11] is avoided by selecting a clone, which can only produce one species of antibodies. By this procedure one can obtain antibodies which are much more specific than those produced by the elegant method of Fitzpatrick and Bundy [17] who employed the metabolically stable PGE_2 analogue, 9-deoxy-9-methylene-$PGF_{2\alpha}$.

Finally, it should be mentioned that we are now in a position to produce specific antibodies of a uniform standard in almost unlimited quantities. By feeding our hybridomas with labelled amino acids we can produce labelled antibodies which should prove particularly valuable in assays based on labelled antibodies instead of labelled antigens [26]. This option together with the relative ease of purifying monoclonal antibodies from culture supernatants is even likely to initiate the displacement of conventional RIAs by assays based on purified antibodies labelled with isotopes, fluorescence markers or enzymes.

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REFERENCES

[1] Kuehl, F.A. and Egan, R.W. (1980) Science 210, 978–984.
[2] Jaffe, B.M., Smith, J.W., Newton, W.T. and Parker, C.W. (1971) Science 171, 494–496.
[3] Levine, L., Gutierrez Cernosek, R.M. and Van Vunakis, H. (1971) J. Biol. Chem. 246, 6782–6785.
[4] Yu, S.C., Chang, L. and Burke, G. (1972) J. Clin. Invest. 51, 1038–1042.
[5] Zusman, R.M., Caldwell, B.V., Speroff, L. and Behrman, H.R. (1972) Prostaglandins 2, 41–47.
[6] Stylos, W.A. and Rivetz, B. (1972) Prostaglandins 2, 103–113.
[7] Kirton, K.T., Cornette, J.G. and Barr, K.L. (1972) Biochem. Biophys. Res. Commun. 47, 903–909.
[8] Caldwell, B.V., Burstein, S., Broch, W.A. and Speroff, L. (1971) J. Clin. Endocrinol. 33, 171–178.
[9] Orczyk, G.P. and Behrman, H.R. (1972) Prostaglandins 1, 3–13.
[10] Gutierrez Cernosek, R.M., Morril, L.M. and Levine, L. (1972) Prostaglandins 1, 71–80.
[11] Jobke, A., Peskar, B.A. and Peskar, B.M. (1973) FEBS Lett. 37, 192–196.
[12] Raz, A., Schwarzman, M., Kenig-Wakshal, R. and Perl, E. (1975) Eur. J. Biochem. 53, 145–150.
[13] Christensen, P. and Leyssac, P.P. (1976) Prostaglandins 11, 399–420.
[14] Horton, E., Jones, R., Thompson, G. and Poyser, N. (1971) Ann. NY Acad. Sci. 180, 351–362.
[15] Polet, H. and Levine, L. (1975) J. Biol. Chem. 250, 351–357.
[16] Dray, F., Charbonnel, B. and Maclouf, J. (1975) Eur. J. Clin. Invest. 5, 311–318.
[17] Fitzpatrick, F.A. and Bundy, G.L. (1978) Proc. Natl. Acad. Sci. USA 75, 2689–2693.
[18] Dray, F., Mana, S. and Bizzini, B. (1982) Methods Enzymol. 86, 258–269.
[19] Köhler, G. and Milstein, G. (1975) Nature 256, 495–497.
[20] Brune, K., Busse, W.D., Grützmann, R: and Wyss, T. (1982) Abstract Book, 5th Int. Conference on Prostaglandins, Florence, May 18–21.
[21] Zusman, R.M. (1972) Prostaglandins 1, 167–168.
[22] Peskar, B.A., Anhut, H., Kröner, E.E. and Peskar, B.M. (1978) Adv. Pharmacol. Ther., 7th Int. Congr. Pharmacol., Paris, 1978, vol.7 (Tillement, J.P. ed.) pp.275–286, Pergamon, Oxford.
[23] Brune, K., Rainsford, K.D., Wagner, K. and Peskar, B.A. (1981) Naunyn-Schmiedeberg's Arch. Pharmacol. 315, 269–276.
[24] Diekmann, J.M., Jobke, A., Peskar, B.A. and Hertting, G. (1977) Naunyn-Schmiedeberg's Arch. Pharmacol. 279, 177–183.
[25] Stähli, G., Staehelin, T., Miggiano, V., Schmidt, J. and Häring, P. (1980) J. Immunol. Methods 32, 297–304.
[26] Ekins, R. (1981) in: Monoclonal Antibodies and Developments in Immunoassay (Albertini, A. and Ekins, R. eds) pp.3–21, Elsevier, Amsterdam, New York.