DEVELOPMENT OF MONOCLONAL ANTIBODIES AND IMMUNOASSAY SYSTEM FOR PRE-SCREENING COPLANAR POLYCHLORINATED BYPHENYLS

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Introduction

A class of coplanar polychlorinated biphenyls (co-PCBs) is known as dioxins. Conventional methods for the analysis of dioxins using high resolution gas chromatography/mass spectrometry (GC/MS), are expensive and time consuming and require specialize equipments. Therefore, alternative simple and cost-effective methods for the determination of these compounds is highly desired. One of the methods that may satisfy these requirements and be an efficient screening tool is an immunoassay method based on poly- or monoclonal antibodies. There are some difficulties in developing an immunoassay for co-PCBs. One major problem is that one antibody cannot detect all the toxic congeners of co-PCBs except for specific congener(s) unlike the GC/MS method. We have targeted the development of an immunoassay system using a number of monoclonal antibodies to co-PCBs that could be used to prescreen for the existence of isomers. It is, therefore, important to select the appropriate congeners to be used in the design and construction of co-PCBs immunoassay. Possible strategies to choose the target measurements by immunoassay include the most *common* congeners[1] or the most *toxic* congeners[2]. According to a survey of dioxins performed by the Ministry of the Environment of Japan[3], 70 to 80% of the total absolute concentration of co-PCBs in soil, fly ash, air, water and other elements, consisted of three congeners. These were 3,3',4,4'-tetrachlorobiphenyl (PCB 77), 2,3,3',4,4'-pentachlorobiphenyl (PCB 105) and 2,3',4,4',5-pentachlorobiphenyl (PCB 118). This is a plausible fact since Japanese commercial PCB preparations (Kanechlor), which have long been considered to be a major source of PCBs in the environment of our country, contained mainly PCB 77 in non-ortho co-PCB[4] and PCB 118 and 105 in mono-ortho co-PCB[5]. On the other hand, the most toxic congeners are 3,3',4,4',5-pentachlorobiphenyl (PCB 126) and 3,3',4,4',5,5'-hexachlorobiphenyl (PCB 169). These congeners have higher toxic equivalency factor (TEF) values, 0.1 and 0.01, respectively. Based on these facts, we decided to target PCB 77, 105 and 118 as the most *common* congeners, and PCB 126 and 169 as the most *toxic* congeners for the synthesis of haptens.

This is the first report of our approach. In this study, we report the production and characterization of monoclonal antibodies (MAbs) to the most common congeners and the most toxic congeners of co-PCB.

Materials and Methods

Synthesis of haptens.

Three types of hapten (Fig. 1) were prepared according to the modified methods of Ya-Wen Chiu *et al.* In accordance with the target congeners, hapten **1** and **2** were designed for the most common congeners, and hapten **3** for the most toxic ones. In all of the haptens, ether-linked spacer was introduced to the 4-position of the polychlorinated biphenyls substituted for chlorine atom.

Production of hybridomas and preparation of monoclonal antibodies.

Each hapten was coupled to KLH (keyhole limpet hemocyanin) as carrier protein by the active ester method. Hapten's carboxyl groups were first activated with carbodiimide and *N*-hydroxysuccinimide in dimethylformamide, then coupled to KLH in borate buffer (pH 9.4). The hapten-KLH conjugates were used as immunogens after removing the unreacted haptens by dialysis. BALB/c mice were immunized by subcutaneous injections of hapten-KLH conjugates with Ribi adjuvant. The development of anti-hapten response in sera were confirmed by ELISA with the haptens coupled to bovine serum albumin (BSA). On day 4 after the intravenous injection, immune spleen cells were fused with myeloma cells (P3X63/Ag8.653 or P3NS-1/Ag4.1) using 50% polyethylene glycol (mw: 1500) and maintained in Dulbecco's modified Eagle's medium supplemented with 15% fetal calf serum. Hybridomas were selected in the same medium containing HAT solution (10^{-4} M hypoxanthine, 4 x 10^{-7} M aminopterin and 10^{-5} M thymidine) and screened by ELISA with hapten-BSA (the first screening). Positive wells of hybridoma in the first screening were further screened by competitive ELISA with free haptens (the second screening). Each well that showed competitively binding response to free hapten was expanded to culture dishes, and then cloned by limiting dilution. MAbs were tested for competitive binding to co-PCB congeners by competitive ELISA. Immunoglobulin subclass was determined by a commercially available typing kit.

Enzyme-linked immunosorbent assay (ELISA).

Each well of 96-well microtiterplates was coated with hapten-BSA. Wells were blocked with phosphate buffered saline (PBS) containing 1% BSA at 4 degree C overnight. A sample, dilution of antiserum for titer experiments or culture supernatant for the first screening of hybridoma, was added to each well and then incubated. For competitive ELISA, a mixture containing free hapten or co-PCB standard in 50%-DMSO-PBS, and the culture supernatant was allowed to react. Peroxidase-conjugated rabbit antibody against mouse IgG was added to each well. After each of

the aforementioned steps, washing was performed five times with PBS containing 0.05% Tween 20. Finally, substrate solution containing *o*-phenylenediamine was added to each well. The color that developed color was measured at 490nm with a microplate reader.

Results and Discussion

Screening of hybridomas.

Subjecting each of three hapten-KLH conjugates to immunization in mice, all mice raised strong anti-hapten responses (signal at serum dilutions > 100,000). Several mice were selected for each immunizing hapten and sacrificed for hybridoma production. After two steps of screening of hybridoma and subsequent cloning, two cell lines which produced desired MAbs, designated I-A and I-B, were established from hapten **1** immunized mice. By the same procedures, four MAbs (designated II-A, II-B, II-C and II-D) were derived from hapten **2** immunized mice. From hapten **3** immunized mice, only one MAb, designated III-A, proved to show competitive binding to co-PCB. All MAbs were found to have IgG_1 class.

Antibody characterization (Cross-reactivity of coplanar PCB congeners).

The cross-reactivity in each MAb was evaluated for 12 co-PCB congeners. For determination of cross-reactivity, the IC_{50} values of PCB 77, 105 and 169 were assigned a value of 100% for the MAb I group (I-A and I-B), II group (II-A, B, C and D) and MAb III-A, respectively. The cross-reactivities for each co-PCB congeners were described as ratios between the IC_{50} value of each congener and that of the target congener. The MAb I-A showed the strongest recognition of PCB 118 (140%), followed by PCB 77 (100%). Whereas in PCB 126, 105, 114, 156, moderate cross-reactivities were observed, namely, 11%, 26%, 9% and 12%, respectively. MAb I-B was found to show similar cross-reactivities to those of MAb I-A. In contrast, 4 antibodies of the MAb II group recognized only one congener, PCB 105. MAb III-A strongly recognized PCB 126 (100%) and 169 (33%), followed by weak responses to PCB 77 and 81.

MAb I groups had good recognition of PCB 118 and PCB 77, whereas MAb II groups were specific to PCB 105. Since these three congeners are the most common congeners, it was suggested that the MAb I and II groups might be a good tool for the estimation of the *absolute* concentration of co-PCB congeners in environmental samples. On the other hand, from the viewpoint of toxic equivalent (*TEQ*) concentration, these three congeners are less important because the most common congeners are not the most toxic ones. According to TEQ concentration, MAb III-A might be a good indicator since this antibody recognized the most toxic congeners PCB 126 and PCB 169.

Acknowledgments

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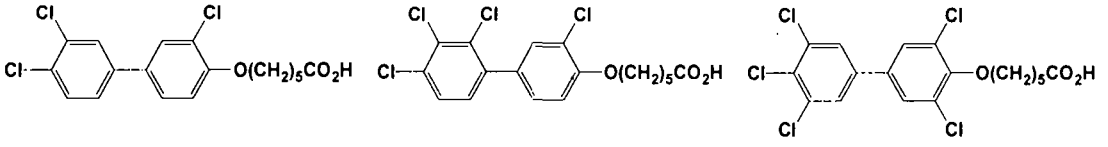

hapten **1** hapten **2** hapten **3**

Figure 1. Structures of haptens for coplanar PCB