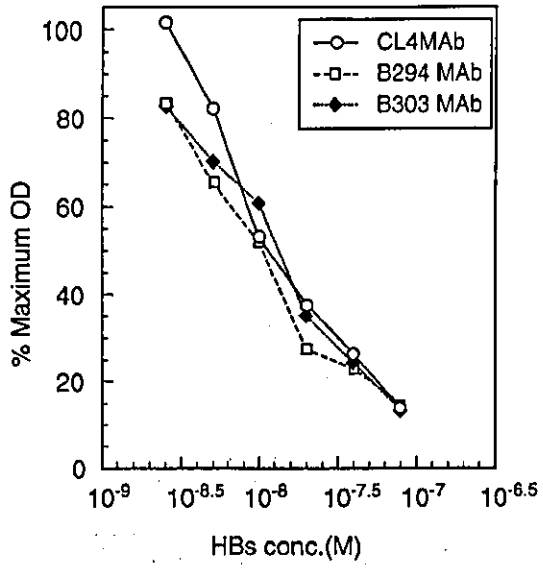

Fig. 5. Inhibition ELISA assays of purified antibodies. Affinities of the CL4, B294, and B303 MAbs were estimated by inhibition ELISA. The HBsAg was used as solid-phase antigen and serially diluted soluble HBsAg was used as inhibitor. The concentration of HBsAg, which gave 50% inhibition of maximum binding reflected MAb affinity, which is approximately the same as the binding constant. The typical result of five experiments is shown.

used as negative control [Masuho et al., 1987]. These cells were clearly labeled by HBIg, CL4, and B303 MAbs (Fig. 6). When the concentration of antibodies was increased to 40 μg/ml for CL4Mab or 20 μg/ml for MAb B303, the FACS profile was altered to a greater extent than when the HBIg concentration was increased to 20 IU/ml.

Complement-Dependent Cytotoxicity

It has been reported that anti-HBs antibodies have complement-dependent cytotoxicity in cells expressing HBs antigens on their surface [Shouval et al., 1982]. To confirm that the Fc domains of plant produced MAbs are functionally equivalent to those of HBIg, complement-dependent cytotoxicity test were done. When Alexander cells were reacted with the parental CL4Mab, MAb B303, positive control HBIg, or negative control TI23 MAb in the presence of rabbit complement, a dose-dependent increase in cytolysis was observed with all anti-HBs antibodies tested, but not with the T123 negative control (Fig. 7). There were no significant differences between the human antibodies and the plant-produced antibody for the complement binding function of the Fc region.

DISCUSSION

This is the report of the production in plant cells of a full-length human IgG that was synthesized using the gene encoding an HBV neutralizing MAb. As such, the plant derived MAb would be expected to be fully active in neutralizing HBV in vivo. The quantities of antibody

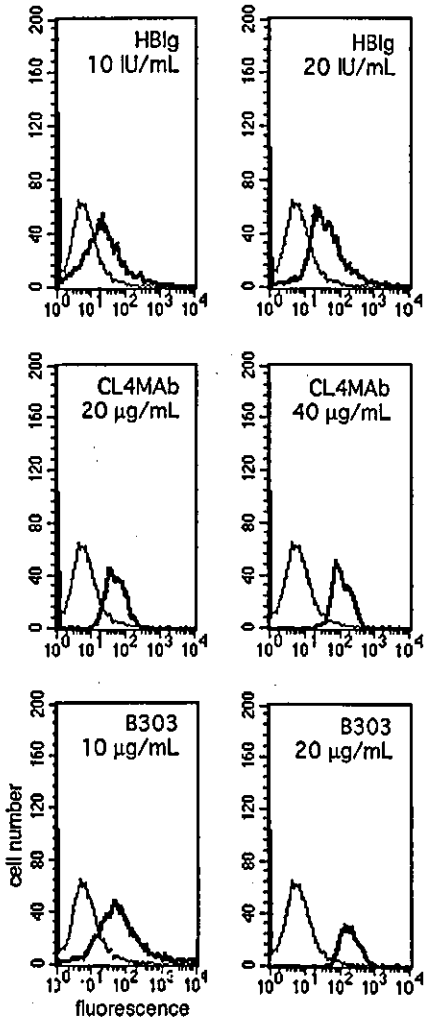

Fig. 6. Binding assay of the antibodies to cell surface HBsAg. The Alexander cells with surface HBsAgs were treated with HBIg, CL4, B303, and TI23 MAbs. Cell-antibody complexes were detected with FITC-conjugated goat anti-human IgG specific antibody and analyzed by flow cytometry. The number of cells are shown on the vertical and immunofluorescence intensity is shown on the horizontal axis. The result of the TI23 MAb (20 μg/ml) treatment is overlaid on each graph as negative control and is shown with a thin line. The typical result of five experiments are shown.

produced were consistent with levels that would be expected from a plant production system [Conrad and Fiedler, 1998]. The average yield of the recombinant protein in our system using plant SS (B303) was usually 0.1–2% of TSP. The MAbs were expressed using three types of signals; specifically, human derived LS, dicotyledonous calreticulin derived SS, and monocotyledonous hordothionin derived SS. The use of hordothionin derived SS transgenic tobacco cells did not consistently result in expression levels of antibody that were sufficient for preparative scale purification. Overexpression of some proteins, when the monocotyledonous signal peptide was used or the processing site of the SS was not correct, were burden for the protein secretion pathway and

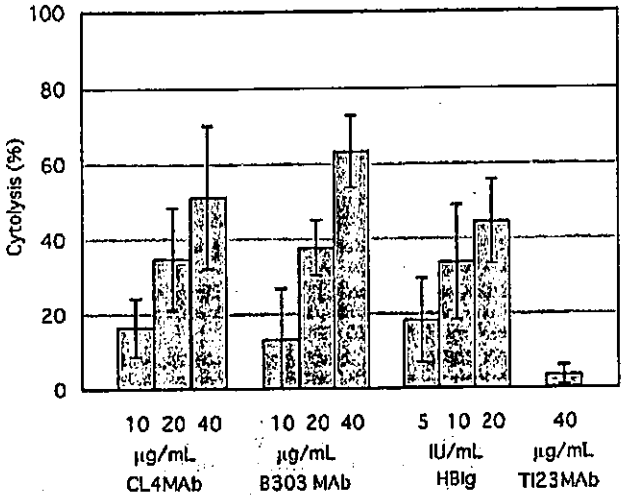

Fig. 7. Antibody induced complement-dependent cytotoxicity. The Alexander cells were treated with CL4 and B303 MAb. Hepatitis B immunoglobulin (HBIg) was used as positive control, and TI23 MAb was used as negative control. Columns and vertical bars represent the mean cytotoxicity values ± SD (n = 3).

resulted in less-than-optimum cell culture conditions. The transgenic tobacco cells containing dicotyledonous SS grew more vigorously and expressed the antibodies more consistently than cells made transgenic with human LS. The human derived signals that originate from the immunoglobulin leader sequence have usually been used for production of antibodies in transgenic plants because it is thought that the correct antibody processing is dependent upon the signal peptide. However, plant derived signal peptide was more suitable for both overexpression of the protein and stable growth of the host culture. Similar results have been reported for the stable expression of the HBs antigen in tobacco cells by plant signal peptide [Sojikul et al., 2003]. When culture conditions and the purification protocols are optimized, yields of full-length recombinant antibodies in tobacco suspension culture were 25 mg/L culture [Fischer et al., 2003]. In addition, when transgenic plants are used as bioreactors as an alternative to suspension cultures, it is easy to scale-up production simply by enlargement of the field area for plant growth.

Even though the transgenic expression systems provide an attractive vision for a source of plant derived MAbs for clinical use, there are problems that need to be solved before implementation of this strategy. Since HBIgs require intravenous injection to prevent the transmission of HBV from mothers to infants, recombinant antibodies used as substitutes for HBIg cannot be antigenic in humans. There are some differences in the protein glycosylation patterns between human and plants. However, studies of mice injected with a recombinant mouse IgG isolated from plants showed that, despite some differences in the glycan groups present on the recombinant antibody, neither the antibody nor the glycans were immunogenic [Chargelegue et al., 2000]. If necessary, the human type of glycosylated antibodies can be expressed in plants. The key technology for transgenic plant production of humanized glycosylated protein has been reported. The introduction of human galactosyltransferase (GalT) into plant cells results in the production of recombinant antibodies with human type β1,4-galactose residues [Palacpac et al., 1999; Bakker, 2001]. The next step for humanization of the glycosylation pattern will be to reduce the plant specific glycan residues, α1,3-fucose and β1,2-xylose. This may be possible by depression of glycosylation enzymes by inhibitory technologies such as RNA interference. Considering the progress in technologies for transgenic plants, many monoclonal antibodies would be produced by plant. Actually, several antibodies produced in plants are now undergoing phase II clinical trials [Fischer et al., 2003].

In this report, we showed that the B303 HBs antibody produced in plants had almost the same affinity and physiological activity as the virus neutralizing parental human monoclonal antibody CL4Mab. The MAb B303 has potential for both prevention and therapy of HBV infection. Characterization of the B294 and B303 MAbs supports the conclusion that these recombinant MAbs derived from the CL4MAb will be a clinical immunotherapeutic for preventing HBV transmission. However, data for CL4MAb or B303 have been obtained using sdr subtype HBV or HBs, and adw subtype HBs expressing cells. The problem of HBV escape mutants following HBV passive immunotherapy points to the need for further analysis of the MAbs against escape mutants. The most common mutations in the HBs protein were at amino acid positions 143–145. These positions are usually essential for recognition of antigen and neutralization by HBs antibodies [Mahoney, 1999]. In vitro studies of the HBV mutants indicated that some polyclonal antibodies more effectively inhibit the secretion of a mutant HBV from infected cells than MAbs [Schilling et al., 2003]. Preparations of polyclonal antibodies are composed of antibodies to single epitope determinants, much like MAbs. It is likely that the combination of some selected MAbs will be an effective means of neutralizing HBV in vivo. At this time, a combination of two MAbs to HBV is being developed as an anti-HBV drug [Galun et al., 2002]. It is necessary to obtain the cDNAs of other MAbs to HBs to produce the MAb cocktail, including CL4MAb, which will be a real substitute of HBIg. It would be desirable to produce the MAb cocktail in the GalT transformed plants. If this is possible, a safe alternative to HBIg will become available. We are confident that the transgenic plant derived biopharmaceuticals will become both safe and economical for promotion of global health.

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