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Influence of subclass-specific anti-idiotypic antibodies on the kinetics of the immune response to BCG

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Summary. The role of anti-idiotypic subclass-specific antibodies was analysed in the regulation of the immune response to BCG in the guinea-pig. The idiotypes to BCG were separated into subclasses and anti-idiotypes were carried out by immunizing with the purified IgG1 and IgG2 anti-idiotypes. The *in vivo* T cell response was recorded by tuberculin skin testing, and the *in vitro* response by lymphocyte stimulation testing with tuberculin. A suppressive effect was detected in cases where the animals were preimmunized with anti-idiotypic IgG2 against anti-BCG IgG1.

In the B cell response, the anti-BCG IgG1 and IgG2 subclasses were also quantified by a solid-phase radioimmunoassay. There were 10 times more IgG2 antibodies than IgG1 against BCG in the guinea-pig, and this major idiotypic subclass was suppressed by the IgG2 anti-idiotype raised against anti-BCG IgG2. The minor component anti-BCG IgG1 was slightly stimulated by both IgG2 anti-idiotypes.

INTRODUCTION

In recent years the potential role of idiotype and anti-idiotype interactions has received considerable attention. Jerne (1971) proposed in the network theory that anti-idiotypic antibodies occupy a key position in the regulation of the immune response. Auto anti-idiotypic antibodies were detected during the primary and secondary immune responses to thymus-dependent and thymus-independent antigens by hapten augmentation of plaque formation (Goidl *et al.*, 1980).

The phosphorylcholine-specific delayed immune response against the phosphorylcholine-binding myeloma protein (TEPC 15) could be depressed by anti-idiotypic antibodies (Yamamoto, Nonaka & Katz, 1979). In this system suppression was only achieved in the induction phase but in other systems suppression was observed in the effector phase also.

Suppression has also been induced by coupling idiotypic antibodies to syngeneic spleen cells (Sy *et al.*, 1979a,b, 1980).

The influence of subclass-specific anti-idiotypic antibodies on the regulation of the immune response was first described by Eichmann (1974, 1975) and Hetzelberger & Eichmann (1978). In their study only the IgG2 subclass of guinea-pig anti-idiotypic antibodies prepared against A5A antibodies of A/J mouse strain suppressed an idiotype which was associated with the antibodies to group A streptococcal carbohydrates.

In this paper we describe both the IgG1 and IgG2 humoral and the cellular immune responses to subclass-specific isologous anti-*Mycobacterium bovis* strain BCG in a homologous immune system of inbred guinea-pigs.

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MATERIALS AND METHODS

Preparation of the anti-guinea pig IgG1 and IgG2 rabbit antibody affinity chromatography columns

Three guinea-pigs in each group were immunized intramusculary by injecting 2 mg bovine serum albumin in Freund's complete adjuvat and/or in Freund's incomplete adjuvant, respectively. An initial booster injection of 1 mg bovine serum albumin in 1 ml saline was given s.c. 4 weeks later, and subsequent booster injections were administered at 2-week intervals. Blood was taken by heart puncture every alternate week. Sera were pooled and stored at −20° (Stewart-Tull, Arbuthnott & Freer, 1975). All sera were treated this way. IgG was prepared by protein A affinity chromatography (Hjelm, Hjelm & Sjöquist, 1972) on CH-Sepharose 4B (Pharmacia Fine Chemicals, Uppsala). IgG1 and IgG2 were isolated by DEAE ion-exchange chromatography (Oliveira *et al.*, 1970) on Sephacel (Pharmacia) by repeated gradient elution with 0·5 M saline in the starting buffer. Only immunoelectrophoretically pure IgG1 and IgG2 fractions from the guinea pig were used for immunizing rabbits and for preparing guinea-pig IgG1- and IgG2-CH-Sepharose 4B affinity columns (Kümel, Daus & Mauch, 1979). Three rabbits in each group were immunized i.m. with guinea-pig IgG1 and IgG2, respectively, in Freund's incomplete adjuvant. After boosting, blood was taken from the ear vein. Immunization and bleeding were alternately repeated until enough serum was obtained.

The two types of sera (anti-IgG1 and anti-IgG2) were purified on guinea-pig IgG1 and IgG2 affinity columns, respectively. The purified rabbit anti-guinea-pig IgG1 and IgG2 antibodies were coupled to CH-Sepharose 4B.

Production of idiotypic and anti-idiotypic subclass-specific antibodies against BCG in the strain 2 guinea pig

Idiotypes. Five guinea-pigs were immunized against fractionated *Mycobacterium bovis* strain BCG Copenhagen (from the Robert Koch Institut, Berlin) homogenate (Mauch & Bremer, 1982). One milligram of BCG homogenate in Freund's incomplete adjuvant was injected i.m. After 4 weeks the animals were boosted s.c., and 3 days later they were bled by heart puncture. Subsequently they were either boosted or puctured once a week. The anti-BCG antibodies were separated on a BCG-CH-Sepharose 4B affinity column. The purified anti-BCG antibodies were further separated into IgG1 ad IgG2 as follows. They were first applied to a rabbit anti-guinea-pig IgG2 affinity column and then to a rabbit anti-guinea-pig IgG1 affinity column (both were prepared as described below) connected in series to the first one. Pure IgG1 was eluted by 0·1 M pH 2·5 glycine from the second column and pure guinea-pig anti-BCG IgG2 was also separately eluted from the first affinity column. The eluates were neutralized and concentrated to 10 mg/ml. The purity of the fractions was established in a solid-phase radioimmunoassay.

Anti-idiotypes. Three guinea-pigs in each group were immunized i.m. by injecting anti-BCG IgG1 and anti-BCG (0·2 mg in Freund's incomplete adjuvant), respectively. Further immunizations and bleedings took place as described above. The various purification steps produced the following preparations:

idiotypes—anti-BCG IgG1, and anti-BCG IgG2;

anti-idiotypes—anti-idiotypic IgG1 against anti-BCG IgG1, anti-idiotypic IgG1 against anti-BCG IgG2, anti-idiotypic IgG2 against anti-BCG IgG1, and anti-idiotypic IgG2 against anti-BCG IgG2.

All of the anti-idiotypic fractions were assessed for idiotype-binding capacity by an indirect radioprecipitin test (Eichmann, 1973). The subclass specificities were controlled in a solid-phase radioimmunoassay (Mauch & Kümel, 1979).

Suppression experiments

Immunizing schedule. Three micrograms of anti-idiotypic antibodies from each group were administered i.v. to the animals (three in each group) 3, 2 and 1 day before starting with the BCG application. BCG immunization began on day 1 with 1 mg injection i.m. or s.c. This was repeated on day 10.

Experiments on T cell suppression. Flank skin tests in preimmunized (see above) guinea-pigs: intradermal tests were performed on both shaved flanks with tuberculin 100 GT (Behring, FRG) on the 34th and 150th days of the suppression experiments. Twenty-four hours later measurements were made of the erythema (by ruler), and the intensity of induration was detected (by a caliper Oditest, Schleuchtern, FRG).

Cell cultures and in vitro *stimulation with tuberculin.* After the second *in vivo* tuberculin test (see above), spleen cell suspensions were prepared as described by Ford (1978). Preparative separation of spleen cells into IgG^+ ad IgG^- lymphocytes was done on goat anti-

guinea-pig immunoglobulin-coated petri-dishes (Mage, McHugh & Rothstein, 1977). The viable lymphocytes (trypan blue exclusion over 97%) were suspended in RPMI-1640 medium containing 10% foetal calf serum (2×10^6 cells/ml). Separation of macrophages from the peritoneal exudate of non-immunized guinea-pigs, and mitomycin C treatment were performed according to Waldron, Horn & Rosenthal (1973). Lymphocyte stimulation tests were performed in quadriplicate in the above mentioned medium. 0·1 ml of lymphocytes and 0·1 of mitomycin C-treated macrophages in RPMI-1640 medium (2×10^6 cells/ml) were incubated with 20 mg/ml tuberculin on microtitre plates (Costar 3696) at 37° in 6% CO_2 atmosphere for 96 hr. To determine the newly synthezised DNA, each cup received a 16-hr pulse of 2 μCi of [^{3}H]-thymidine (New England Nuclear). Cells were harvested by a cell harvester (Dynatech, Nurtingen, FRG), and radioactivity was measured in a Hewlett Packard scintillation counter.

Experiments on B cell suppression. Antibody titres to BCG were measured six times within the first 50 days of suppression in a solid-phase radioimmunoassay (Mauch & Kümel, 1979). Briefly, single microtitre cups (Dynatech) coated with 0·1 mg/ml BCG were used to incubate the sera. ^{125}I-labelled rabbit anti-guinea-pig IgG1 and IgG2 antibodies were used to quantify the antigen–antibody complexes in the sera. Bound radioactivity was counted in a gamma-ray spectrometer (Kontron, MR 252, München). Each assay was performed in triplicate.

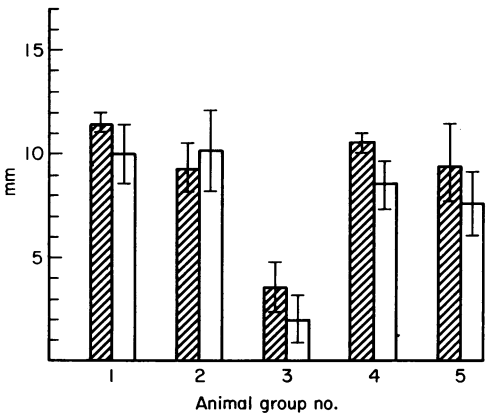

Figure 1. Tuberculin skin tests on the 34th day of the suppression experiment. Erythema formation (*hatched bars*), thickness of induration (*open bars*) measured in mm 24 hr after injecting with 100 GT. Groups 1–5 represent the animal groups in the suppression experiment: (1) control; (2) preimmunized with anti-idiotypic IgG1 against anti-BCG IgG1; (3) preimmunized with anti-idiotypic IgG2 against anti-BCG IgG1; (4) preimmunized with anti-idiotypic IgG1 against anti-BCG IgG2; (5) preimmunized with anti-idiotypic IgG2 against anti-BCG IgG2.

RESULTS

Isolation of IgG by protein A affinity chromatography produced a serum-free IgG fraction in large quantity. The immunoelectrophoretic pattern of IgG1 and IgG2 Sephacel ion-exchange chromatography showed no contamination of serum proteins. Rabbit anti-guinea-pig IgG1 and IgG2 fractions were cross-adsorbed by affinity chromatography. The results of immunoelectrophoresis and radioimmunoassay demonstrated that they were highly pure and specific against the guinea-pig subclass-specific antibodies (data not shown).

In vivo T cell suppression in time course

Figures 1 and 2 show the erythema formation and the thickness of induration 24 hr after injecting 100 GT tuberculin to the guinea-pigs in the suppression experiment on the 34th and 150th days.

Among the four groups of animals immunized with the four groups of anti-idiotype preparations, only the anti-idiotypic IgG2 antibodies against anti-BCG IgG1 showed a significant suppression compared to the control group (control means that the animals were not preimmunized with anti-idiotypic antibodies before the BCG immunizing schedule started). This suppression could already be demonstrated 34 days

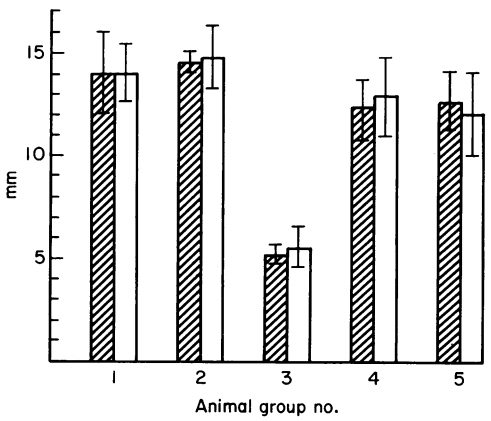

Figure 2. Tuberculin skin tests on the 150th day of the suppression experiment. For details, see legend to Fig. 1.

after BCG immunization (Fig. 1), and was still detectable on the 150th day (Fig. 2). However, the mixed anti-idiotypes had no suppressive effect at all (data not shown).

In vivo T cell response

Figure 3 shows the results of the lymphocyte stimulation test on spleen lymphocytes prepared from the animals on the 150th day of the suppression experiment.

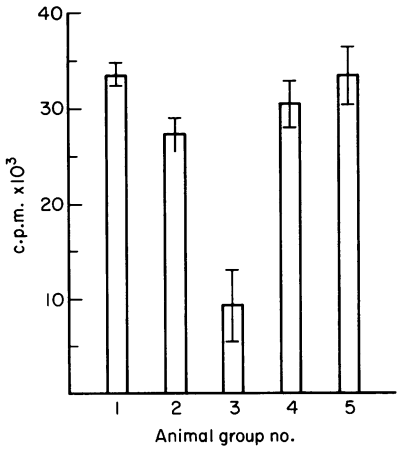

Figure 3. Lymphocyte stimulation test with tuberculin on the 150th day of the suppression experiment. Bars represent [^{3}H]-thymidine incorporation per 10^6 lymphocytes. For details of animal groups 1–5, see legend to Fig. 1.

Compared to the control group only the anti-idiotypic IgG2 antibodies against anti-BCG IgG1 caused suppression; none of the others had any effect on the immune response to BCG in the guinea-pig.

B cell response in the suppression experiment

The B cell response could be analyzed in more detail since, in the solid-phase radioimmunoassay, the idiotypic subclasses to BCG could be separately measured by radioactive labelled anti-guinea-pig IgG1 and IgG2. The IgG1 immune response to BCG is demonstrated in Fig. 4.

The antibody levels were observed in four preimmunized groups plus control in 8-day intervals. The anti-idiotypic antibodies of the IgG2 subclasses had a significant stimulatory effect on the IgG1 immune response to BCG. Both of the IgG1 anti-idiotypes had only a small suppressive effect.

The IgG2 immune response to BCG (Fig. 5) was more complex. In our experiments there were 10 times more IgG2 anti-BCG antibodies than IgG1. Surprisingly, only the anti-idiotypic IgG2 antibodies produced against anti-BCG IgG2 had a suppressive effect on the IgG2 immune response to BCG. Whereas the T cell immune response could only be suppressed by the anti-idiotypic IgG2 produced against anti-BCG IgG1

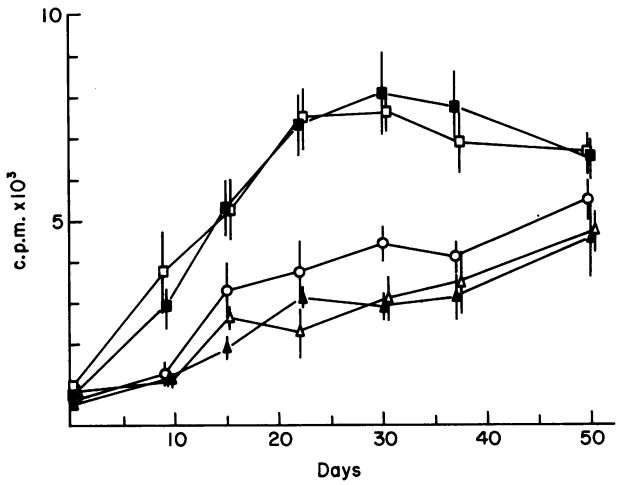

Figure 4. IgG1 B cell response to BCG during the first 50 days of suppression detected by a solid-phase radioimmunoassay. (○) Control; (△) preimmunized with anti-idiotypic IgG1 against anti-BCG IgG1; (▲) preimmunized with anti-idiotypic IgG1 against anti-BCG IgG2; (□) preimmunized with anti-idiotypic IgG2 against anti-BCG IgG2; (■) preimmunized with anti-idiotypic IgG2 against anti-BCG IgG1.

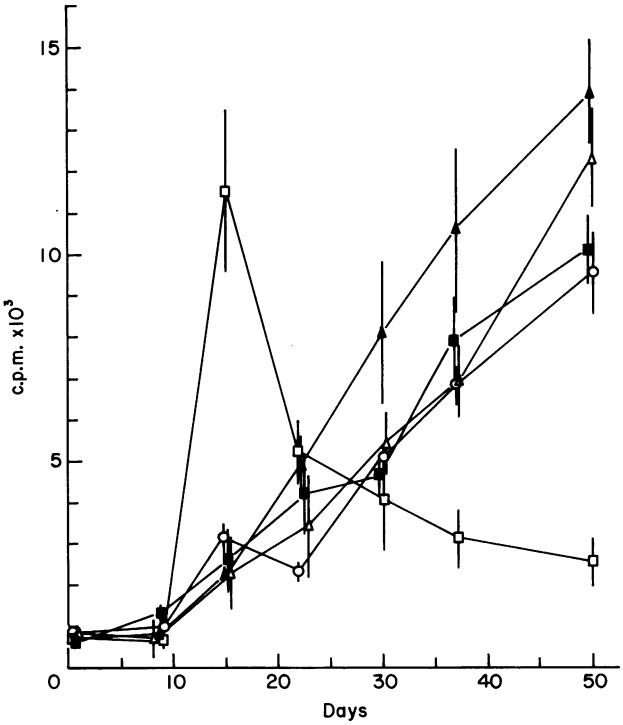

Figure 5. IgG2 B cell response to BCG during the first 50 days of suppression detected by a solid-phase radioimmunoassay. For symbols, see legend to Fig. 4.

antibodies. Both anti-idiotypic IgG1 subclasses raised against anti-BCG idiotypes stimulated the BCG immune response.

DISCUSSION

In this study, we attempted to clarify the mechanism of T and B cell immune responses to BCG after administration of isologous anti-idiotypic subclass-specific antibodies. The immunosuppressive effect of anti-idiotypes is well known (Schrier *et al.*, 1982; Sternick *et al.*, 1981), but we are not aware of previous studies on guinea-pigs. In a study of BCG-anergy in mice, Schrier *et al.* (1982) were able to demonstrate a role for genes linked to the immunoglobulin heavy chain complex (Igh). In another experiment, it was shown that, at a cellular level, responses to BCG were T cell-dependent (Sternick *et al.*, 1981). Furthermore, Kato & Yamamoto (1982) produced non-specific macrophage-induced suppression of delayed-type hypersensitivity in mice by previous i.v. injection of BCG cell wall. In our study, suppression of the T cell response in the guinea-pig was also T cell-dependent because only unprimed mitomycin C-treated macrophages were used in the *in vitro* experiments. Moreover, we could demonstrate a selective subclass-specific immune suppression on the BCG delayed-type hypersensitivity in the guinea-pig *in vivo* by skin tests, and anti-idiotypic antibodies of the IgG2 subclass prepared against IgG1 anti-BCG suppressed the immune response to BCG. It is interesting that the mixed anti-idiotype did not provoke any response. The anti-BCG IgG2 antibodies were 10 times more effective than IgG1 in modulating the role of anti-idiotypes in the B cell immune response. A minor IgG1 immune response was stimulated by anti-idiotypic IgG2 antibodies produced either against idiotypic IgG1 or IgG2; whereas the primary idiotypic IgG2 antibodies could only be suppressed by anti-idiotypic IgG2 antibodies produced against IgG2 antibodies to BCG. The other preparations had little or no stimulating effect.

Differences in the role of anti-idiotypic subclasses on idiotypic subclasses or on the two types of immune

response against BCG suggest that there may be two populations of suppressor lymphocytes; one of them reacts with the T cells and the other one with the B cells. These populations should recognize private antigenic structures on the idiotypes or on the T lymphocytes. Further studies on the isolation of different T suppressor cell populations which might show different patterns of suppression on T and B cells will be of considerable interest.

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