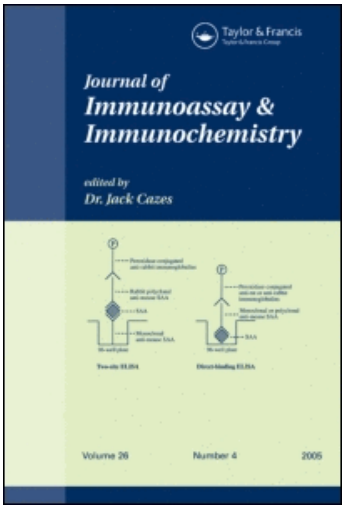

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ANTI-IDIOTYPIC SERA AGAINST MONOCLONAL ANTI-PORCINE GROWTH HORMONE ANTIBODIES: PRODUCTION IN RABBITS AND CHARACTERIZATION OF SPECIFICITY

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ABSTRACT

Antisera were raised in rabbits against two mouse monoclonal anti-porcine growth hormone (pGH) antibodies. Anti-idiotypic antibodies were isolated from rabbit sera by successive passage over immunoadsorbent columns of normal mouse Ig (mIg), followed by the specific immunizing monoclonal and elution from the latter. Enzyme linked immunoadsorbent assay (ELISA) for anti-idiotype and free idiotype were established. While showing specificity for their respective inducing monoclonals, anti-idiotypes also cross reacted in varying degrees with other anti-pGH monoclonals regardless of specificity differences between the antibodies, demonstrating the presence of a cross-reactive idiotype.

KEY WORDS: Anti-idiotypic antibodies ; growth hormone

INTRODUCTION

Idiotypes (Id) are phenotypic markers of B and T cell receptor V-region genes (1). Production of Id and auto anti-id by the same individual during a normal immune response has been demonstrated and is thought to be part of an immunoregulatory network (2,3). Although the significance of Id-anti-Id interactions in immunoregulation is still debated, manipulation of the immune system by injecting Id (Ab1) or anti-idiotypic antibodies (Ab2) is normally

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accepted. Monoclonal antibodies to GH have been used in the generation of both polyclonal and monoclonal anti-idiotypic antibodies (4,5,6). Some of these inducing monoclonals or Ab1 have been found to be enhancing to the biological functions of GH and furthermore their corresponding anti-idiotypes can mimic the actions of GH through GH/receptor interactions (6). Polyclonal anti-idiotypic antisera to rat GH antibodies capable of binding to GH receptors and increasing weight gain in hypophysectomised rats have been described (4). Anti-idiotypic antibodies produced in lactating cows by immunization with rabbit antibodies against bovine somatotropin (bST) have been isolated with biological effects similar to bST (5). In addition a mouse monoclonal anti pGH anti-idiotype has been described which is conformation dependent and was shown to promote growth in the hypophysectomised rat model (6).

Anti-idiotypic antibodies are useful reagents in analysis of panels of antibodies of a defined antigenic specificity and can help define the structural basis for antigen binding and the molecular genetic origin of the antibodies. Described here are two rabbit anti-idiotypic antisera against monoclonal anti pGH of defined specificity. The anti-idiotypes are used to define idiotypic relationships between a panel of anti-pGH MAbs and to detect a particular idiotype in serum of mice immunized with GH. Analysis of the panel of anti-pGH MAbs suggests the presence of a cross-reactive idiotype.

MATERIALS AND METHODS

Monoclonal antibodies

Anti-pGH MAbs 1/197, 3/24, 5/2, 5/22, 5/32 and 5/39 derived from fusions 1 to 5 from 5 separately pGH immunized mice The specificities of the purified anti-pGH MAbs for GH of different species and for individual GH epitopes were studied by direct binding ELISA (7). (Table 1). The following hormones were used to coat microtiter wells (Dynatch Labs, Alex. VA) at 50 μg/ml (50 μl): porcine GH (Intervet); ovine GH, (NIH GH-S-11); bovine GH (NIH B-18); equine GH (8); human GH (1st International Standard for Immunoassay 66/217, NIBSC, Hampstead, London); rat GH (NIDDK rGH-B-12); porcine PRL (USDA B1-1); ovine PRL (NIH-P-S-9); human PL (biological grade) was a gift from Dr. S. Raiti (Hormone Distribution Office, NIH, NIAMDD, Bethesda, MD, USA). Overlapping peptides were derived from the N-terminus of porcine GH (1A, 1C) and equine GH (1B) (gift from Dr. R. B. Heap) with the following sequences:

Pep 1A: Pro-Ala-Met-Pro-Leu-Ser-Ser-Leu-Phe-Ala-Cys
Pep 1B: Phe-Pro-Ala-Met-Pro-Leu-Ser-Ser-Leu-Phe-Ala-Cys
Pep 1C: Ala-Phe-Pro-Ala-Met-Pro-Leu-Ser-Ser-Leu-Phe-Ala-Cys

MAbs were titrated in a 3-fold dilution series at a starting concentration of 10 μg/ml against the respective hormones. Bound antibody was detected by horseradish peroxidase (HRP)-conjugated rabbit anti-mouse IgG (ICN Biomedical Inc. U.K.) giving an O.D. 450 in the ELISA of between 0.8 -1.5.

Immunization of rabbits with anti-pGH MAbs

Female New Zealand White (NZW) rabbits were purchased from Morton Rabbits, Stansted, Essex. Rabbits received 200 μg of purified mouse anti pGH IgG1 MAbs, 3/24 or 5/39 emulsified in complete Freund's adjuvant by injection into the hind legs; inoculations were repeated three times at monthly intervals, the boosting dose being 100 μg per animal in incomplete Freund's adjuvant. Bleeds from rabbits were taken 2 weeks after each inoculation. Sera were assayed for reactivity by ELISA against the respective MAbs in the presence of normal mouse Ig (P3) to block anti-Ig activity.

Production and test of anti-idiotypic antibodies

Normal mouse Ig (mIg) and purified MAbs were each coupled to activated Sepharose 4B (Pharmacia, Uppsala, Sweden). Normal mIg was the fraction precipitated from BALB/c mouse serum at 18% sodium sulphate. Coupling of all proteins was at a ratio of 10 mg protein per g of Sepharose using the recommended conditions.

Hyperimmune rabbit serum was passed three times through a Sepharose column (3g) coupled to normal mIg to remove anti-Ig activity. Bound Ig was eluted with 200 mM glycine/HCL buffer, pH 2.5. The effluent of the column after three passages was shown to be highly enriched for anti-idiotype and to contain only a trace amount of anti-Ig. To complete the purification, the anti-id was passed twice through an affinity column of Sepharose 4B coupled to either anti-pGH mAb 5/39 or MAb 3/24. Specific antibody against MAb 5/39 or MAb 3/24 was eluted with glycine/HCL, pH 2.5 and its specificity demonstrated by ELISA. The IgG1 BALB/c mouse myeloma protein P3 was used as a specificity control for monitoring purification of anti-idiotype.

Anti-Idiotype assay

For the detection of anti-idiotype the wells of a 96 well microtitre plate were coated with a solution of the purified anti-pGH MAb (5/39 or 3/24) (50 μg/ml, 100 μl per well) in 0.1M NaHCo3 buffer, pH 8.3 overnight at 4°C. Wells of control plates were also coated with a non-idiotypic, IgG1, MAb P3. All wells were washed with PBS containing 5% fetal calf serum (FCS), 0.05% Tween 20, followed by blocking for 1 hr with 10% FCS in PBS and dried. Hyperimmune rabbit serum (anti-idiotype) or purified anti-idiotype was then titrated (3-fold series, in 10% FCS/ PBS, 100 μl per well). Plates were incubated for 1 hr at RT, washed three times and dried. Donkey anti-rabbit Ig horseradish peroxidase conjugate (Serotec, U.K; 1:2000, 50 μl) was added to each well and incubated for 1 hr. Plates were washed three times and the assay developed by the addition of substrate, 150 μl (tetramethylbenzidine in dimethylsulfoxide, 1.5 mg/ml, 15 μl H_2O_2 (6% v/v) and 13.5 ml H_2O) for 5 min. The reaction was stopped by the addition of 30 μl of 2M H_2SO_4 and colour detected at O.D. 450. The sensitivity of this assay was 1 ng and the intra assay and inter assay co efficients of variation were 7% and 12% respectively.

Cross-Reactivity of purified anti-idiotypes with a panel of anti-pGH MAbs

MAbs 3/24, 5/39, 5/32, 5/22, 5/2 and 1/197 were coated separately, as described above onto microtiter plate wells and the ability of purified anti-idiotypes 5/39 and anti 3/24 to bind to the panel was assessed. A 3-fold dilution series of purified anti-idiotype was incubated for 1 h on the MAb coated ELISA plates, followed by washing and incubation with a donkey anti-rabbit IgHRP conjugate as above.

Inhibition of binding of anti pGH MAbs to pGH by anti-idiotype

To determine the extent of the inhibition of MAb binding to pGH by anti-idiotype, MAbs 5/39 or 3/24 were titrated in the pGH coated wells in a 3-fold dilution series respectively in 10% FCS/PBS, in the presence or absence of preimmune sera or purified rabbit anti-idiotype (10 μg). The plates were incubated for 1 hr at RT, washed four times and dried. This was followed by the addition of

anti-mouse Ig HRP conjugate at 4°C for 1 hr. The assay was developed as above. The percentage inhibition in each well was calculated by comparison with control wells receiving no anti-idiotype.

RESULTS

Epitope specificity of anti-pGH MAbs

Anti pGH MAbs have been described and their bioactivity in the dwarf mouse bioassay assessed (7). A summary of the cross-reactivity of each MAb against a panel of GHs, PRLs and PL of various species is shown in Table 1.

MAb 1/197 is strongly cross-reactive with all GHs, PRLs and human PL, but non-reactive with the N-terminal GH peptides.

MAb 3/24 is strongly cross-reactive with porcine, equine and rat GH, weakly cross-reactive with ruminant and human GH and non-reactive with the GH N-terminal peptides, PRL or PL

MAb 5/2 is strongly cross-reactive with porcine and equine GHs and weakly with rat GH.

MAb 5/22 reacts uniqely with porcine GH.

MAb 5/32 is cross-reactive only with porcine and equine GH

MAb 5/39 is selectively non-reactive with ovine or human GH, but otherwise as for MAb 3/24.

Purification of rabbit anti-idiotypes against monoclonal anti pGH MAb 5/39

Stages in the purification of anti 5/39 idiotype were monitored by ELISA against MAb 5/39 or the IgG 1 myeloma protein P3 coated onto the surface of microtitre wells. (Figure 1 A,B). Titrations are shown and demonstrate significant anti-idiotype activity in the hyperimmune sera. Absorbsion of the rabbit antiserum with Sepharose-conjugated normal mIg removed most of the binding activity for P3, but only partially reduced binding to MAb5/39. Absorbsion and elution from a Sepharose-coupled MAb 5/39 column enabled the isolation of anti-idiotype which bound specifically to the MAb 5/39 -treated wells and hardly to P3 coated wells. The anti-id containing fraction bound to MAb 5/39 some 10, 000 times better than to P3. The yield of purified anti-5/39 idiotype was 70 μg/ml of serum. The isolation of anti-idiotypes against MAb 3/24 followed a similar pattern.

Table 1

GROUPING OF ANTI PGH MABS ACCORDING TO CROSS REACTIVITY

Mab	CLASS	P	O	B	E	H	R	1A	1B	1C	pP	oP	hPL
1/197	IgM	++	++	++	++	++	++	-	-	-	++	++	++
3/24	IgG1	++	-	+	++	-	++	-	-	-	-	-	-
5/2	IgG1	++	-	-	++	-	+	-	-	-	-	-	-
5/22	IgG1	++	-	-	-	-	-	-	-	-	-	-	-
5/32	IgG1	++	-	-	+	-	-	-	-	-	-	-	-
5/39	IgG1	++	-	++	++	-	++	-	-	-	-	-	-

P: porcine GH; O: ovine GH; B: bovine GH; E: equine GH; H: human GH; R: rat GH.
1A, 1B, 1C: N-terminal peptides of GH described in materials and methods.
pP, porcine prolactin; oP, ovine prolactin; hPL, human placental lactogen
++ (100%); + (40-70%); - (0%) when compared to the O.D. 450 of pGH at 1 μg of MAb.

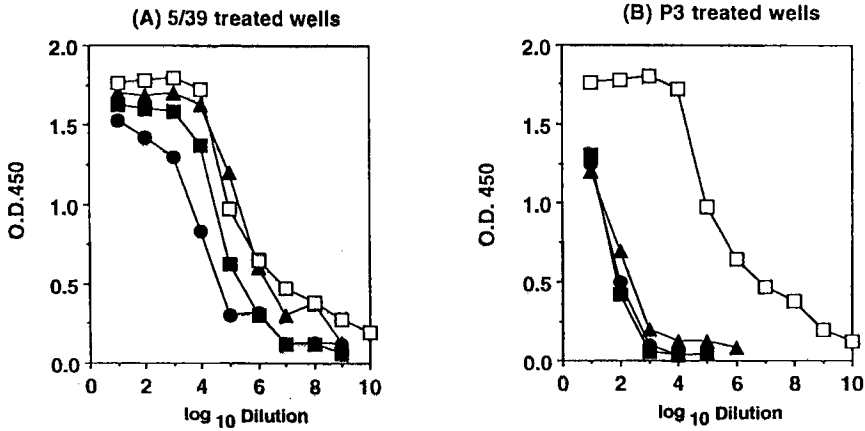

Figure 1
Purification from rabbit serum of anti-idiotype against the 5/39 monoclonal anti growth hormone. Antibody binding to MAb 5/39 (A) and to the mouse myeloma IgG 1 protein P3 (B). Anti-idiotypes were assayed at various steps in the purification procedure. The preparations assayed were the rabbit hyperimmune serum (□), rabbit Ig fraction after passage through Sepharose coupled normal mIg adsorbent (■), rabbit Ig after passage through a 5/39 idiotype absorbent (▲) and rabbit Ig eluted from the 5/39 adsorbent (●). This is the anti-idiotype.

Inhibition of binding of anti pGH MAbs to GH by anti-idiotype.

The ability of both purified anti-idiotypes to interfere with binding of anti pGH MAbs to pGH was tested by titrating MAbs 3/24 and 5/39 against pGH coated ELISA plates in the presence or absence of purified specific anti-idiotype. The presence of anti-5/39 idiotype (10 μg) in the titration of MAb 5/39 against pGH was sufficient to completely block the binding of aproximately 10 ng of MAb 5/39. (Figure 2A). Therefore this anti-idiotype may not be as specific for the pGH combining site of MAb 5/39 as is the anti-24 idiotype for the pGH combining site of MAb 3/24. Anti 3/24 idiotype (10 μg) was sufficient to completely block the binding of 1 μg of MAb 3/24 to pGH with preimmune sera having littlle or no affect (Figure 2B).

Cross reactivity of anti-idiotypes with anti-pGH MAbs.

Purified anti-idiotypes, anti-3/24 and anti-5/39 were tested for binding to various members of the anti pGH panel (Table 1) by direct binding to MAb coated microtitre wells (Figure 3). Each anti-idiotype showed a high degree of specificity for its own idiotype but the cross reactions were significant. Anti-5/39-idiotype cross-reacted with MAbs 5/22 and 5/2 with a titre equivalent to that of MAb 5/39 and cross reacted with MAb 5/32 with a titre of about 12% of the reaction of MAb 5/39 itself. Binding to MAbs 3/24 and 1/197 was less than 0.05% (Figure 3A). Binding to the non specific myeloma protein P3 was negligible for both anti-idiotypes (data not shown). Anti-3/24 idiotype was the most cross reactive, binding to MAb 1/197 with a titre equivalent to MAb 3/24, 12.5% to MAb 5/2 and 6% to MAbs 5/32, 5/22 and 5/39 (Figure 3B). Both anti-idiotypes appear to divide the MAbs into two groups, namely those which were obtained in fusion 5 i.e. MAbs 5/39, 5/32, 5/22 and 5/2 and were therefore derived from the same mouse and those which came from other fusions (1 and 3, 1/197 and 3/24). This phenomena appears to occur regardless of the specificity differences between the antibodies. (Table 1). i.e. anti 5/39 idiotype cross-reacted strongly with MAbs from fusion 5 (IgG only) each with a different specificity for the panel of GHs, and weak cross reactions for MAbs from fusions 1 and 3 (IgM and IgG). Anti-3/24 idiotype cross reacted strongly with all the MAbs, however it bound more strongly to MAbs from fusions 1 and 3 and had weaker cross reactivity for MAbs derived from fusion 5.

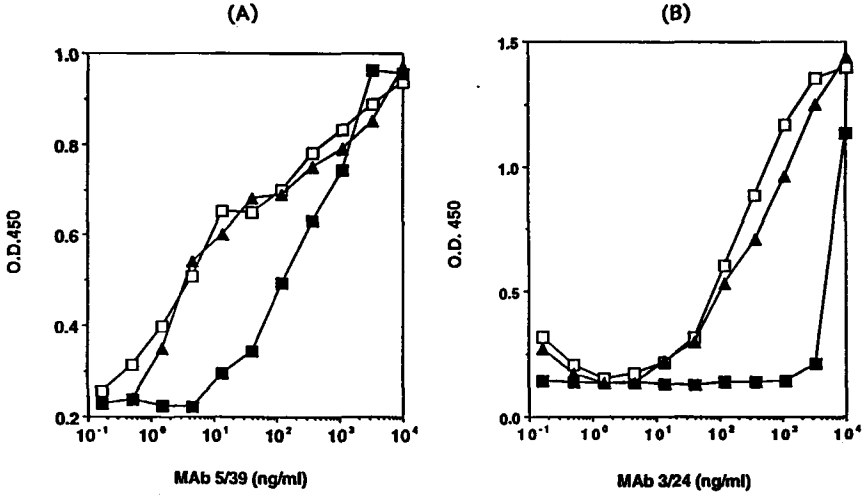

Figure 2
Specific inhibition of binding of MAb 5/39 (A) and MAb 3/24 (B) to pGH by their respective anti-idiotypes. Monoclonal antibodies were titrated in the wells in the presence of anti idiotype (10 μg) (■) assay diluent (□) or preimmune sera (▲) as controls.

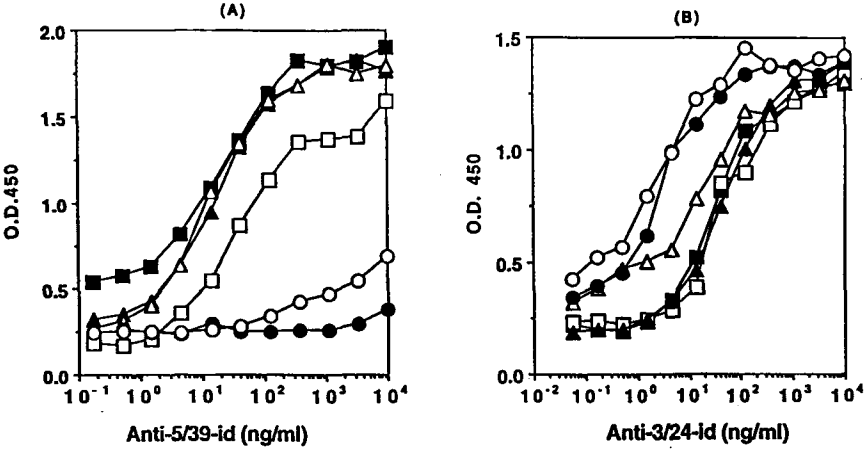

Figure 3
Cross reactivity of rabbit anti-idiotypes with mouse monoclonal anti-pGH antibodies. The purified rabbit anti-idiotypes, anti-5/39 (A) anti-3/24 (B) were titrated in the ELISA against the five IgG1 monoclonal anti-pGH antibodies, 3/24 (●); 5/39 (■); 5/32 (□); 5/22 (▲); 5/2 (□); and the IgM monoclonal 1/197 (○).

DISCUSSION

Polyclonal anti-idiotypic antisera were raised against two anti pGH MAbs of a panel of anti-pGH MAbs (MAbs 3/24 and 5/39). These MAbs originated from different fusions and had a different specificity patterns against the panel of hormones tested. Relatedness among the panel was demonstrated by the strong cross-reactions of the anti-idiotypes with members of the panel of MAbs and a correlation between cross-reactivity pattern and mouse origin of the immunizing MAb. To judge from the reduction in binding titre of MAb 3/24 to pGH coated wells, by the presence of anti-idiotype, 30 % of these may be binding site directed. By contrast less than 10% of anti-5/39-idiotype is likely to be binding site directed. Anti-idiotypic antibodies against anti GH previously described (4) were AB2 beta types against the antigen combining site (9). They bound to the GH receptor in vitro and were capable of inducing body weight gain and increasing IGF 1 concentrations in serum. These were referred to as "hormone mimics".

The wide cross-reactivity pattern of the anti-idiotypes against the panel of MAbs, despite differences in epitope specificity suggests the presence of a cross-reactive idiotype. Cross-reactive anti-idiotypes are known to occur on antibodies of many individuals responding to the same antigen and occur repeatedly because they are encoded by germ line V genes (9). Cross-reactive anti-idiotypes may have clinical application in the treatment of GH deficiency where immunological intolerance to GH results in the production of high titred, high affinity anti-GH antibodies with the ability to neutralize GH (10). Apart from the possibility of substituting GH deficient patients with IGF, one might also consider ways to overcome immunological intolerance to GH by the passive administration of anti-idiotypes against a cross reactive idiotype. This approach has been used in the anti-idiotypic suppression of auto antibodies to factor V111 (antihaemophilic factor) (11) and to inhibit the production of anti-DNA antibodies in patients with systemic lupus erythematosus (SLE) (12). Cross-reactive mouse monoclonal anti-idiotype have also been used to suppress rheumatoid factor synthesis (13). Thus it is possible to suppress the synthesis of a specific idiotypic human autoantibody by the addition of anti-idiotype.

To conclude, we have purified two preparations of rabbit anti-idiotypic sera against anti-pGH MAbs. These have been used to detect a likely cross-reactive idiotype in a panel of anti-pGH MAbs. Sequence analysis of these MAbs will

enable us to define the germ line genes involved in the expression of this idiotype. Future studies will address their biological function in *in vivo* GH bioassay systems and *in vitro* in GH receptor assays to access their enhancement or neutralization potential on the biological function of GH.

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ABBREVIATIONS

Id: idiotype; pGH: porcine growth hormone; bST: bovine somatotropin; ELISA: enzyme linked immunosorbent assay; PRL: prolactin; PL: placental lactogen; HRP: horse radish peroxidase; Ig: immunoglobulin; MAb: monoclonal antibody; FCS: fetal calf serum; IgF1: insulin like growth factor 1

REFERENCES

1. Bona, C. A. Functional heterogeneity of anti-idiotype antibodies. In Anti-idiotypes, Receptors, and Molecular Mimicry. (Linthicum D. S. and Farid N. R. ed. Springer, New York) 1988; 7-20.

2. Jerne, N. K. Towards a network theory of the immune system. A. Immun. (Ins. Pasteur) 1974; 125C:373-89.

3. Gehra, R. S. Presence of auto-anti-idiotypic antibody during the normal human response to tetanus toxoid antigen. J. Immunol 1982;129: 139-44.

4. Gardner M. J., Morrison C. A.., Stevenson L. Q., and Flint D. J. Production of anti-idiotypic antisera to rat GH antibodies capable of binding to GH receptors and increasing body weight gain in hypophysectomized rats. J. Endocrinol 1990; 125, 53-59.

5. Schalla, C.L., Roberge, S., Wilkie, B.N., McBride, B.W. and Walton J. S. Production of anti-idiotypic antibodies resembling bovine somatotropin by active immunization of lactating cows. J. Endocrinol. 1994; 141(2), 203-8.

6. Wang, B. S., Zhang, R. J., Bona, C. A., and Moran, T. M. Promotion of animal growth with a monoclonal anti-idiotypic specific to anti-porcine growth hormone antibody. Mol. Immunol. 1994; 31(9), 651-656.

7. Cahill, C. M., Holder, A. T., Lawton, T. L., Butcher, G. W., and Taussig, M. J. Recognition of porcine growth hormone by a panel of monoclonal antibodies. Hybridoma 1997; 16(4): 371-9.

8. Cahill C. M. Van der Kohl, H., Goode, J. A. and Hayden T.J. Development of homologous radioimmunoassays for equine GH and equine PRL and their application to the detection of circulating levels of hormone in horse plasma. Reprod Nutri Dev 1994; 34: 309-28.

9. Taussig M. J. Anti-idiotypic antibodies and their uses. Animal Cell Biotechnology 1990; V 4 343-77

10. Schwarz, S., Berger, P., Frisch, H., Moncayo, R., Phillips, J. A., and Wick, G. Growth hormone blocking antibodies in a patient with deletion of the GH-N gene. Clin Endocrinol, 1987; 27: 213-24.

11. Sultan, Y., Maisonneuve, P., Kazatchkine, M. D. and Nydegger, U. E. Anti-idiotypic suppression of autoantibodies to factor 8 (antihaemophilic factor) by high dose intravenous gammaglobulin. Lancet 1984; 2: 765-8.

12. Epstein, A., Greenberg, M., Diamond, B. and Grayzel, A. I. Suppression of anti-DNA Antibody synthesis in vitro by a cross-reactive anti-idiotypic antibody. J Clin Invest 1987; 79, 997-1000.

13. Takeuchi, T., Hosono, O., Koide, J., Homma, M. and Abe. T. Suppression of rheumatoid factor synthesis by anti-idiotypic antibodies in rheumatoid arthritis patients with cross-reactive idiotypes. Arthritis Rheum. 1985; 28: 873-81.