THE EFFECT OF PURIFIED ALPHA 1 ANTITRYPSIN ON PWM DRIVEN IgG SYNTHESIS

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SUMMARY   As part of a study investigating the mechanisms for the association of alpha 1 antitrypsin (alpha 1 AT) deficiency with immune disorders, the effect of purified alpha 1 AT on PWM driven IgG synthesis of peripheral blood lymphocytes was investigated. This demonstrated that alpha 1 AT in physiological doses enhanced IgG synthesis in stimulated but not resting cells. This effect is probably due to the known inhibition by alpha 1 AT of macrophage function. Increasing number of macrophages are known to inhibit IgG synthesis in the PWM system suggesting that this apparently paradoxical enhancement by alpha 1 AT is probably due to inhibition of these cells.

Introduction

In a study of the immune abnormalities that may pre-dispose subjects with alpha 1 antitrypsin (alpha 1 AT) deficiency to immune mediated inflammatory diseases such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA) and anterior uveitis (AU) we have previously demonstrated a variety of abnormalities including the macrophage mediated suppression of PHA induced proliferation of mononuclear cells. In that study we were unable to demonstrate any effect of alpha 1 AT on PWM induced lymphocyte proliferation. We have now extended these observations by investigating the effect of alpha 1 AT on *in vitro* PWM IgG synthesis. Our results indicate an apparently paradoxical enhancement of IgG synthesis by purified alpha 1 AT.

Materials and Methods

(a) Alpha 1 Antitrypsin

This was purified essentially according to the method of Laurell (1) which utilizes the affinity of alpha 1 AT for 3-carboxy-4-nitrobenzen-ethiol modified glutathione sepharose (pharmacia fine chemicals, Uppsala, Sweden). Extensive immunoabsorption with specific antisera including rabbit anti-human albumin, prealbumin, immunoglobulin and alpha 1 antichymotrypsin (dako corp., Santa Barbara, USA) removed small quantities of remaining impurities. Alpha 1 AT thus purified exhibited normal microheterogeneity with isoelectric focusing on polyacrylamide gels at pH 3·5–5 and normal antitryptic activity utilizing the method of Dietz (2). Immunoelectrophoresis of the highly concentrated protein with an antibody to whole serum revealed only a single arc due to alpha 1 AT.

(b) IgG Synthesis

This was carried out essentially as previously described (3). Mononuclear cells (MN) were isolated using density gradient centrifugation

then washed 6 times in Hanks Balanced Salt Solution containing 10% foetal calf serum (FCS). The cells were then prepared at a concentration of 4×10^6/ml in RPMI 1640 containing 10 mmol/ml glutamine (flow laboratories, Sydney, Australia), 0·05% gentamycin (essex cemie ag, Lucerne, Switzerland) and 20% FCS (Batch No. 074, australian laboratories services, Rockdale, Australia). This rather than human serum was used, as the assay system involved a radioimmunoassay (RIA) for human IgG. Calf IgG does not cross react with human IgG and hence would not interfere with the RIA is present. FCS also contains no alpha 1 AT although it may well have another protein with equivalent function.

100 µl aliquots of the cell suspension were dispersed into flat bottom microtitre plates (flow laboratories inc., McLean, Virginia, USA) and 100 µl of PWM (Batch No. 15K0201, gibco, Grand Island, NY, USA) in RPMI at a dilution of 1/20 and 1/200 was added. The cells were then cultured for 8 days and the amount of IgG produced in each well was assessed with an RIA as previously described (3).

Briefly the amounts of IgG produced were measured in the culture fluids by double antibody inhibition radioimmunoassay. The amounts of IgG in a standard normal serum (DAC) was calibrated from the WHO reference standard (NHS 16/86) and the Australian standard reference serum for immunoglobulins (ASPS 78/1). The assay detected from 2,000 to 8 ng/ml of culture fluid. Appropriate dilutions of the culture supernatants were selected to obtain values corresponding to the steepest linear part of the standard radioimmunoassay curve. The means ± sem of the amounts of IgG synthesized per well in triplicate cultures were calculated.

### (c) Effect of Purified Alpha 1 AT on IgG Synthesis

MN cells from 3 alpha 1 AT deficient patients and 11 controls were cultured with purified alpha 1 AT at dosages of 0–8 g/l in the FCS supplement and IgG synthesis assessed as outlined earlier. Human serum could not be employed as a part of this system because of the constraints of the IgG RIA.

Results

The effect of purified alpha 1 AT on PWM induced IgG synthesis was estimated in 14 subjects. In four of these no

FIG. 1. The effect of the addition of purified alpha 1 AT (4 g/l of serum supplement) on PWM driven IgG synthesis in 10 subjects. The left diagram displays the absolute amounts synthesized and the right demonstrates the percentage change in synthesis after addition of the alpha 1 AT.
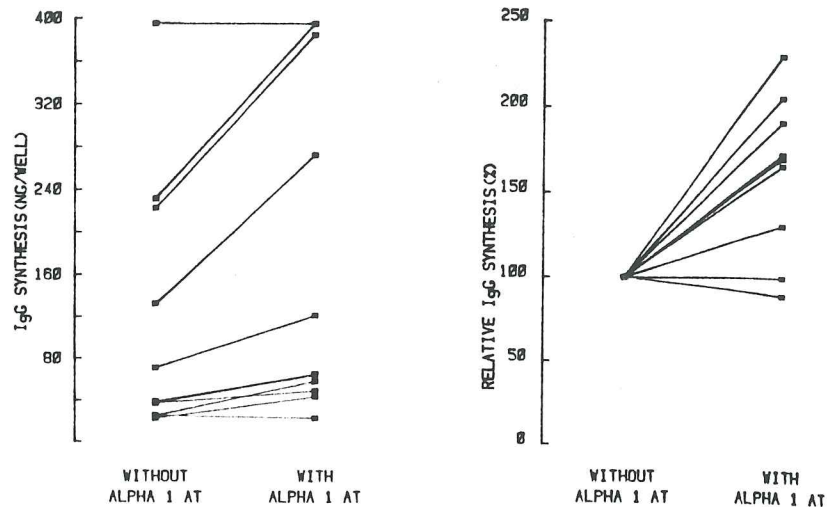
IgG synthesis occurred with PWM stimulation. The remaining 10 subjects (7 normals and 3 alpha 1 AT deficient patients) demonstrated an increase in IgG synthesis (fig. 1) with increasing amounts of alpha 1 AT that was maximal at serum level of about 1–2 g/l (fig. 2) in FCS. This increase was significant ($p < 0.01$) using Wilcoxon's signed ranks test and no similar increase in unstimulated IgG synthesis was found.

DISCUSSION

There have been no previous studies of the effect of alpha 1 AT on IgG synthesis in humans. Studies carried out in mice using *in vitro* and *in vivo* priming with sheep red cells demonstrated that alpha 1 AT inhibited immunoglobulin synthesis (4). Our general hypothesis has been that alpha 1 AT deficient subjects tend to hyperreact due to decreased inhibitory stimuli and this may be the reason for their propensity to develop inflammatory diseases. Our previous studies support this view. Therefore, the effect of alpha 1 AT on IgG synthesis, at first glance, appears paradoxical as it enhanced IgG synthesis of both control and alpha 1 AT deficient lymphocytes. This enhancement is exponential and makes a plateau at alpha 1 AT level in the FCS of about 1–2 g/l being a mirror image of the suppressive effect noted on PHA induced proliferation (5).

This enhancement of IgG synthesis probably largely represents an idiosyncrasy of the PWM system. Unlike
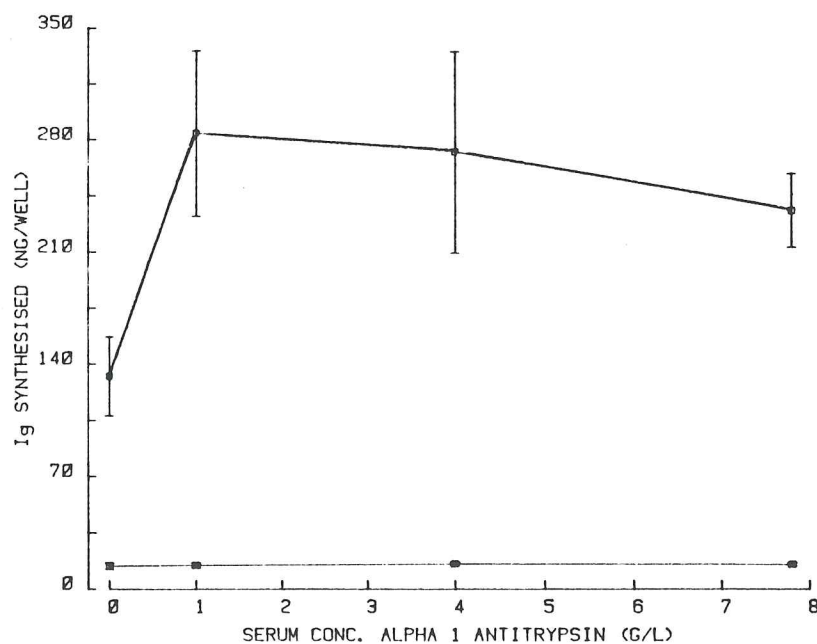

FIG. 2. The effect of increasing serum concentrations of purified alpha 1 AT on IgG synthesis in a single individual. The results are plotted as mean ± 1 standard error. The top curve shows the effect on PWM stimulated cells and the bottom curve on unstimulated cells.

the effect of alpha 1 AT on the PHA response, which is associated with *in vivo* differences in DTH (6), its effect on IgG synthesis is not associated with *in vivo* changes in Ig levels (6). Alpha 1 AT has no effect on PWM induced proliferation (5) and its effect on IgG synthesis is explainable on the basis of its action on adherent cells (AC). The ratio of AC to NAC (non-adherent cells) is known to be critical in PWM induced Ig synthesis. Within broad limits decreasing AC results in enhancement of IgG synthesis (7–9). It has already been shown that alpha 1 AT inhibits AC function resulting in decreased production of a factor enhancing lymphocyte proliferation to PHA, perhaps interleukin 1. If in the PWM system alpha 1 AT inhibited the production of the AC factor normally limiting IgG synthesis, enhancement would result in a similar manner to that seen when reducing AC numbers. In other words, the apparent disparate changes in IgG synthesis and lymphocyte proliferation may simply reflect inhibition of macrophage function with results differing dependent on which of the macrophage factors is dominant and is being measured in the *in vitro* system.

CONCLUSION

Alpha 1 AT, in physiological doses, enhances PWM driven *in vitro* IgG synthesis in both normal and severely alpha 1 AT deficient subjects. This finding is unexpected and is probably an idiosyncrasy of the PWM system which is highly macrophage dependent. Alpha 1 AT is known to inhibit the function of these cells and presumably therefore has the same effect as decreasing macrophage numbers which is known to enhance IgG synthesis.

REFERENCES

1. Laurell, C. B., Pierce, J., Persson, U. and Thulin, E. (1975). Purification of alpha 1 antitrypsin from plasma through thiol-disulphide interchange. *Eur. J. Biochem.*, **57**, 107.
2. Dietz, A. A., Robinstein, H. M. and Hodges, L. (1974). Measurement of alpha 1 antitrypsin in serum by immunodiffusion and by enzymatic assay. *Clin. Chem.*, **20**, 396.
3. Cooper, D. A., Duckett, M., Petts, V. and Penny, R. (1979). Corticosteroid enhancement of immunoglobulin synthesis by pokeweed mitogen stimulated human lymphocytes. *Clin. Exp. Immunol.*, **37**, 145.
4. Arora, P. K., Miller, H. C. and Aronson, H. C. (1978). Alpha 1 antitrypsin is an effector of immunological stasis. *Nature*, **274**, 589.
5. Breit, S. N., Luckhurst, E. and Penny, R. (1983). The effect of alpha 1 antitrypsin on the proliferative response of human peripheral blood lymphocytes. *J. Immunol.*, **130**, 681.
6. Breit, S. N., Robinson, J. P., Luckhurst, E., Clark, P. and Penny, R. (1982). Immunoregulation by alpha 1 antitrypsin. *J. Clin. Lab. Immunol.*, **7**, 127.
7. Knapp, W. and Baumgartner, G. (1978). Monocyte mediated suppression of human B lymphocyte differentiation *in vitro*. *J. Immunol.*, **121**, 1177.
8. Montazeri, G., Chiorazzi, N., Fu, S. M. and Kunkel, H. G. (1980). Regulatory role of circulating monocytes in the differentiation and proliferative response of human B lymphocytes. *Clin. Immunol. Immunopathol.*, **16**, 1.
9. Cooper, D. A., Hansen, P., Duckett, M. and Penny, R. (1981). Glucocorticosteroid enhancement of immunoglobulin synthesis by pokeweed mitogen stimulated human lymphocytes. IV. Role of the monocyte. In *Mechanisms of Lymphocyte Activation* (edited by K. Resch and H. Kirchner), p. 683. Elsevier/North Holland, Amsterdam.