Brief Definitive Report

Complement-mediated Adipocyte Lysis by Nephritic Factor Sera

By Peter W. Mathieson, Reinhard Würzner,* David B. G. Oliveira, Peter J. Lachmann,* and D. Keith Peters

*From the Department of Medicine and the *Medical Research Council Molecular Immunopathology Unit, University of Cambridge, Cambridge CB2 2QQ, UK*

Summary

Recent data indicate a previously unsuspected link between the complement system and adipocyte biology. Murine adipocytes produce key components of the alternative pathway of complement and are able to activate this pathway. This suggested to us an explanation for adipose tissue loss in partial lipodystrophy, a rare human condition usually associated with the immunoglobulin G (IgG) autoantibody nephritic factor (NeF) which leads to enhanced alternative pathway activation in vivo. We hypothesized that in the presence of NeF, there is dysregulated complement activation at the membrane of the adipocyte, leading to adipocyte lysis. Here we show that adipocytes explanted from rat epididymal fat pads are lysed by NeF-containing sera but not by control sera. A similar pattern is seen with IgG fractions of these sera. Adipocyte lysis in the presence of NeF is associated with the generation of fluid-phase terminal complement complexes, the level of which correlates closely with the level of lactate dehydrogenase, a marker of cell lysis. Lysis is abolished by ethylenediaminetetraacetic acid, which chelates divalent cations and prevents complement activation, and reduced by an antibody to factor D, a key component of the alternative pathway. These data provide an explanation for the previously obscure link between NeF and fat cell damage.

The complement system plays an integral role in both afferent and efferent limbs of the immune response, with an important role in host defence (1). Complement activation occurs either via the classical pathway, typically initiated by antigen–antibody complexes, or via the alternative pathway. Both pathways proceed via the third component of complement (C3) to a terminal pathway which leads to lysis of target cells. The key proteins in the alternative pathway are factors D and B. Factor D cleaves factor B complexed with activated C3 to form the alternative pathway C3 convertase, C3bBb. There is continuous low-level activity of the alternative pathway in vivo; this is controlled by the regulatory proteins factors H and I and by the fact that C3bBb is unstable, with a half-life of around 4 min in vitro (2). Nephritic factor (NeF) is a human autoantibody described originally in patients with mesangiocapillary glomerulonephritis type II (3, 4) but associated more consistently with the unusual condition of partial lipodystrophy (PLD) (5). In PLD, there is loss of fat from the face and upper body which is usually of acute onset, often after a viral infection (6). It has been demonstrated that NeF is an IgG autoantibody to the alternative pathway C3 convertase, C3bBb, which it stabilizes against dissociation by factor H, the normal control mechanism. This leads to dysregulation in vivo of the alternative pathway and results in hypocomplementemia with low levels of C3 and the presence of the complement fragment C3dg in the circulation (2, 5).

The mechanism of the association between this pathological activation of the alternative pathway and partial lipodystrophy has been obscure. However, the recent discovery (7, 8) that factor D of the alternative pathway is identical to adipsin, a serine protease of previously unknown function produced by mature adipocytes, has suggested a possible mechanism for the association. Adipocytes have now been shown to produce not only factor D, but also factor B and C3, and to be capable of activating the alternative pathway in vitro and in vivo (9). We have therefore explored the possibility that in the presence of NeF this local production of complement components and particularly of factor D may lead to damage to the adipocyte. We describe here in vitro experiments that show complement-mediated lysis of adipocytes by NeF-containing sera. Release of lactate dehydrogenase (LDH) was measured as a marker of adipocyte lysis (10), and we also quantitated the generation of terminal complement complexes (TCC). The level of fluid-phase TCC correlates closely with TCC assembly on target cells (where TCC forms the membrane attack complex, MAC) and provides a reliable index of terminal complement activation (11).

Materials and Methods

Serum samples were collected from three patients with NeF and from six controls (five normal sera from healthy laboratory staff

 J. Exp. Med. © The Rockefeller University Press • 0022-1007/93/06/1827/05 $2.00
Volume 177 June 1993 1827–1831

and serum from a patient with lupus nephritis and hypocomplementemia as a disease control). Aliquots of fresh serum were frozen at −20°C until use. IgG fractions were prepared either by affinity chromatography on protein G or by 50% ammonium sulphate precipitation.

Epididymal fat pads were removed from Brown Norway rats, minced into small fragments, and digested with type II collagenase (Sigma Immunochemicals, Poole, NK) for 45 min at 37°C. A single cell suspension of adipocytes was obtained by sieving through a sterile nylon sieve before centrifugation at 400 *g* for 1 min. In some experiments, other target cells were used: human adipocytes prepared in a similar way from small samples of subcutaneous fat obtained at routine surgery; the murine preadipocyte cell line F442A (12); and rat peripheral blood mononuclear cells prepared by density gradient centrifugation of anticoagulated whole blood.

All incubations were in Medium-199 (Flow Laboratories, Irvine, UK), supplemented with 20 mM D-glucose (Sigma Immunochemicals) and 100 mg/l L-glutamine (Flow Laboratories), and buffered with 25 mM Hepes (Flow Laboratories), at 37°C in a humidified atmosphere with 5% CO_2. 800 μl of adipocyte suspension was added to 2-ml wells in a tissue culture–treated plate (Corning Inc., Corning, NY), and incubated with 800 μl of serum (normal, disease control, or NeF). In other experiments, a similar quantity of purified IgG was added, together with 400 μl of normal serum as a source of complement components. The effects of EDTA, which chelates divalent cations and abolishes complement activation, and a mAb which binds to and blocks the function of factor D (13) were also examined. Positive controls were incorporated: detergent lysis of adipocytes using 2% Triton X-100 (Sigma Immunochemicals) for maximal LDH release and a concentration of zymosan designed to cause maximal alternative pathway complement activation (15 mg/ml) for TCC generation. 50-μl aliquots of infranatant were removed at intervals and stored at 4°C overnight before being assayed in one batch. All samples were coded and analyzed without knowledge of experimental groups.

LDH assays were performed on an analyzer (Monarch Centrifugal; Instrumentation Laboratory, Warrington, UK) using a colorimetric assay based on the generation of nicotinamide adenine dinucleotide (NAD^+) and lactate from pyruvate and NADH (14).

TCC was assayed by sandwich ELISA as previously described (15). Briefly, mAb (WU 7–2) directed against a neoepitope of TCC was adsorbed to microplate wells (1.5 μg/well, overnight at 4°C). After blocking with PBS containing 1% gelatin, wells were incubated with test samples at 1:16 and 1:80 dilutions. Biotinylated polyclonal goat anti-C6 IgG was then added (2.5 μg/well), followed by horseradish peroxidase–labeled streptavidin (Amersham International, Amersham, Bucks, UK) at a dilution of 1:500. Finally, 2,2'-azino-di-(3-ethylbenzthiazoline) sulfonic acid (Sigma Immunochemicals) was added and the increase in absorption at 410 nm was recorded using an automated ELISA plate reader (Bio-Rad Laboratories, Hemel Hempstead, UK). The data were related to a standard curve calibrated using a standard complement-activated serum with a known concentration of TCC.

Results

Incubation of rat epididymal fat pad cells with NeF-containing sera was consistently associated with progressive LDH releases over 20 h, whereas incubation with control sera was associated with little or no rise in LDH (Fig. 1: results for two NeF sera. We have recently tested a third NeF serum, and this gave a similar pattern of LDH release). By

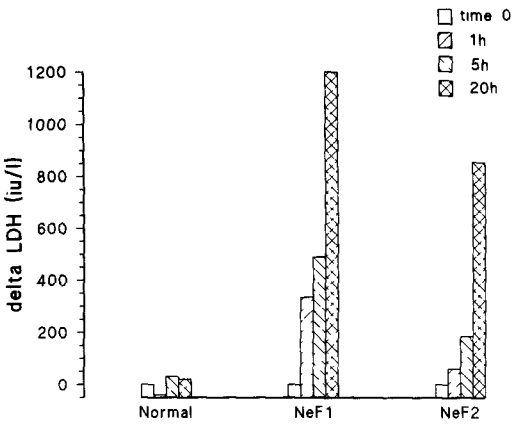

Figure 1. LDH activity (expressed as *delta LDH*, i.e., change from baseline level) with time in infranatants from incubations of adipocytes with one normal and two NeF sera. Data shown is representative of eight similar experiments in which the two NeF sera and six control sera were tested.

20 h, the level of LDH in the incubations with NeF sera approached that seen when cells were totally lysed with detergent. IgG fractions of the sera gave a similar pattern (data not shown). There was a similar effect on human adipocytes, but rat lymphocytes and the murine preadipocyte cell line F442A were not affected, so that there was no evidence of a nonspecific toxic effect (data not shown). NeF sera contained only low levels of TCC initially, but TCC levels rose progressively with time during incubation with adipocytes. There was a smaller rise in TCC with normal sera (Fig. 2). TCC levels correlated closely with LDH release in incubations with NeF sera, but with normal sera there was no correlation (Fig. 3). When zymosan was used to cause maximal complement activation, high levels of TCC were produced, but no rise in LDH occurred (data not shown). LDH release from adipocytes after incubation with NeF sera was abolished by coincubation with EDTA, and was reduced by coincubation with an anti-factor D mAb (Fig. 4).

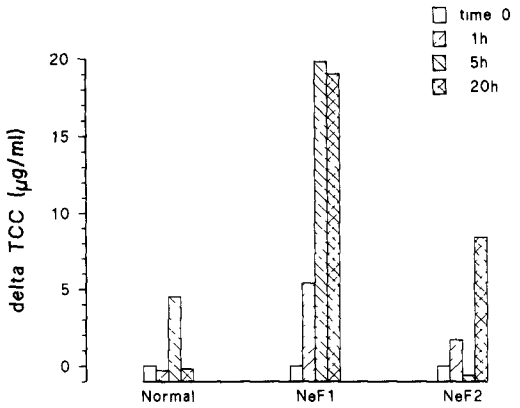

Figure 2. TCC measurements (expressed as *delta TCC*, i.e., change from baseline level) with time from same infranatants as in Fig. 1.

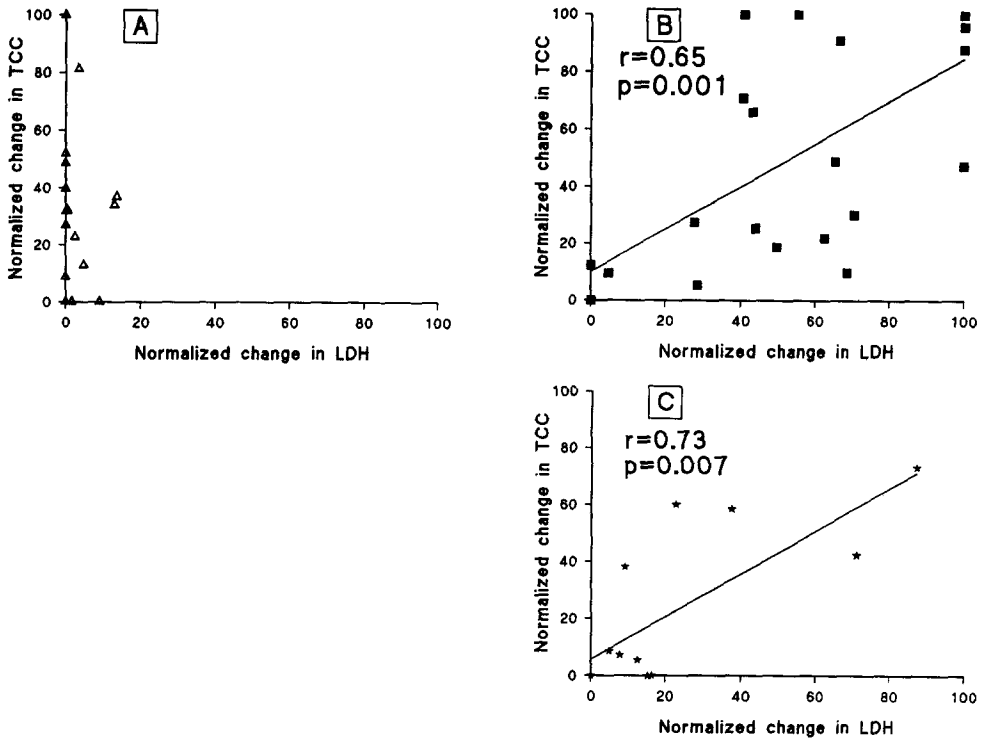

Figure 3. Scatterplot relating changes in LDH and TCC. Data pooled from the four experiments in which LDH and TCC were both measured, normalized by expressing maximum change from baseline in each experiment as 100% and all other results as percentage of this. Data shown for one normal serum (*A*; no significant correlation) and two NeF sera (*B* and *C*; Pearson's *r* as indicated). Other control sera also showed no significant correlation.

Discussion

These data indicate that NeF-containing sera lead to adipocyte lysis by a complement-dependent mechanism. This may explain the long-standing mystery of the link between NeF and PLD. We postulate that local production of factor D and other complement components by the adipocyte becomes deleterious in the presence of NeF, which deregulates the alternative pathway. Baseline levels of TCC in the NeF sera were low, as might be expected from the fact that such sera are hypocomplementemic and functionally complement deficient. However, these sera generated high levels of TCC during incubation with adipocytes, and the level of TCC correlated closely with the level of LDH, a marker of cell lysis. The slopes of the regression lines (Fig. 3) for the two NeF sera were virtually identical, suggesting that the relationship between LDH release and TCC generation was very similar for these two sera. With control sera, by contrast, although some TCC was generated, the consequences were different since there was no adipocyte lysis. Maximal TCC generation with zymosan did not lead to cell lysis. IgG preparations, which retained NeF activity, had a similar effect to whole sera, indicating that the lytic activity was contained in the IgG portion. Blockade of the complement system by EDTA abolished the effect, and a mAb which blocks the function of factor D, reduced the amount of cell lysis. Thus NeF-containing sera have the potential to cause complement-mediated fat cell damage via the alternative pathway.

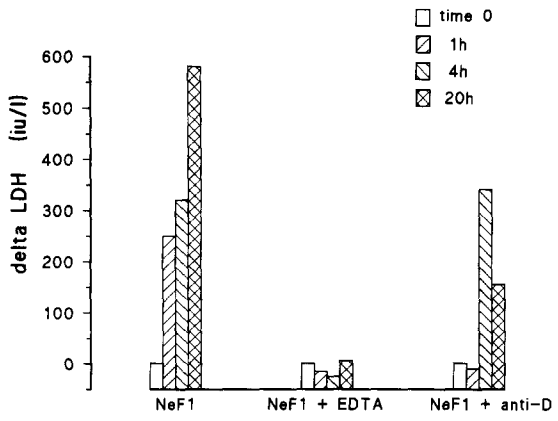

Figure 4. LDH activity (*delta LDH*) with time in infranatants from incubations of adipocytes with NeF sera alone and with the addition of 10 mM EDTA or 200 μg/ml of anti-D antibody.

The production of factor D, factor B, and C3 by adipocytes (7–9) and the capacity of these cells to activate the alternative pathway of complement (9) indicate a previously unsuspected role for the alternative pathway of complement in normal regulation of adipose tissue. Factor D/adipsin expression is increased in fasting states and decreased in certain animal models of obesity (7, 16). Adipocytes from obese animals are less able to activate the alternative pathway in vitro and in vivo (9). Previously, factor D was thought to be solely produced by cells of the monocyte/macrophage lineage, but it is now apparent that adipose tissue is a major site of factor D production in rodents (7) and in humans (8). Deficiency of factor D in humans is rare. Such individuals are not obese (17), so that clearly other mechanisms of adipose tissue regulation must exist.

Improved understanding of regulatory mechanisms in adipose tissue has obvious wide-ranging implications. The complement system, and particularly the alternative pathway, may play a physiological role in adipocyte biology. Our data provide an illustration of the consequences of deregulation in humans. Whether complement-mediated adipocyte lysis could be engaged for therapeutic benefit in obesity remains highly speculative, but is an attractive possibility. Antibodies to adipocytes have been widely tested in animals, with the aim of producing lean animals. This is particularly effective in the rat, where antiadipocyte antibodies reduce body fat by complement-mediated adipocyte lysis (18). The loss of fat tissue in such animals seems to be permanent. The metabolic consequences of this are largely unknown. Fat cell loss in PLD in human is also permanent, suggesting that, as in the animal studies, once adipocytes are lost, they are not replaced. It remains unclear why adipocyte loss in PLD is localized to the upper body, and why adipose tissue is lost suddenly and in only a proportion of NeF-positive individuals. There may be regional and/or individual differences in the expression of complement components and/or regulatory proteins by adipocytes. The association between the onset of PLD and recent viral infection could be explained by an increase in vascular permeability and enhanced access of NeF to the vicinity of the adipocyte, although this would require the phenomenon to be extremely sensitive to NeF concentration. Perhaps more likely is an effect of increased levels of certain cytokines during intercurrent infection: alternative pathway activation by adipocytes is enhanced by IL-1 and TNF-α (9). Further study of adipocyte lysis by NeF sera should enable testing of these hypotheses.

The anti-factor D antibody and cell line F442A were kind gifts from, respectively, Dr. J. Schifferli (Hôpital Cantonal Universitaire, Geneva, Switzerland) and Dr. Howard Green (Harvard Medical School, Boston, MA). We thank the Department of Clinical Biochemistry at Addenbrooke's Hospital for measurements of LDH.

P. W. Mathieson is a Medical Research Council Clinician Scientist Fellow; R. Würzner is supported by the Deutsche Akademische Austauschdienst and the Arthritis & Rheumatism Council; D. B. G. Oliveira is a Lister Institute Research Fellow.

Address correspondence to Dr. P. W. Mathieson, University of Cambridge School of Clinical Medicine, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2SP, UK.

Received for publication 16 February 1993.

References

1. Kinoshita, T. 1991. Biology of complement: the overture. *Immunol. Today.* 12:291.
2. Daha, M.R., D.T. Fearon, and K.F. Austen. 1976. C3 nephritic factor (C3Nef): stabilization of fluid phase and cell-bound alternative pathway convertase. *J. Immunol.* 116:1.
3. Spitzer, R.E., E.H. Vallota, J. Forristal, E. Sudora, A. Stitzel, N.C. Davis, and C.D. West. 1969. Serum C'3 lytic system in patients with glomerulonephritis. *Science (Wash. DC).* 164:436.
4. Varade, W.S., J. Forristal, and C.D. West. 1990. Patterns of complement activation in idiopathic membranoproliferative glomerulonephritis, types I, II, III. *Am. J. Kidney Dis.* 16:196.
5. Sissons, J.G.P., R.J. West, J. Fallows, D.G. Williams, B.J. Boucher, N. Amos, and D.K. Peters. 1976. The complement abnormalities of lipodystrophy. *N. Engl. J. Med.* 294:461.
6. Senior, B., and S.S. Gellis. 1964. The syndromes of total lipodystrophy and of partial lipodystrophy. *Pediatrics.* 33:593.
7. Rosen, B.S., K.S. Cook, J. Yaglom, D.L. Groves, J.E. Volanakis, D. Damm, T. White, and B.M. Spiegelman. 1989. Adipsin and complement factor D activity: an immune-related defect in obesity. *Science (Wash. DC).* 244:1483.
8. Tyler White, R., D. Damm, N. Hancock, B.S. Rosen, B.B. Lowell, P. Usher, J.S. Flier, and B.M. Spiegelman. 1992. Human adipsin is identical to complement factor-D and is expressed at high levels in adipose tissue. *J. Biol. Chem.* 267:9210.
9. Choy, L.N., B.S. Rosen, and B.M. Spiegelman. 1992. Adipsin and an endogenous pathway of complement from adipose cells. *J. Biol. Chem.* 267:12736.
10. Newby, A.C., J.P. Luzio, and C.N. Hales. 1975. The properties and extracellular location of 5'-nucleotidase of the rat fat cell plasma membrane. *Biochem. J.* 146:625.
11. Mollnes, T.E., T. Lea, and J. Tschopp. 1989. Activation-dependent epitopes in the terminal complement pathway. *Complement Inflammation.* 6:223.
12. Djian, P., M. Phillips, and H. Green. 1985. The activation of specific gene transcription in the adipose conversion of 3T3 cells. *J. Cell. Physiol.* 124:554.
13. Pascual, M., E. Catana, F. Spertini, K. Macon, J.E. Volanakis, and J.A. Schifferli. 1990. A monoclonal antibody which blocks the function of factor D of human complement. *J. Immunol. Methods.* 127:263.
14. Neilands, J.B. 1955. Lactic dehydrogenase of heart muscle. *Methods Enzymol.* 1:449.
15. Würzner, R., M. Schulze, L. Happe, A. Franzke, F.A. Bieber, M. Oppermann, and O. Götze. 1991. Inhibition of terminal complement complex formation and cell lysis by monoclonal antibodies. *Complement Inflammation.* 8:328.
16. Flier, J.S., K.S. Cook, P. Usher, and B.M. Spiegelman. 1987. Severely impaired adipsin expression in genetic and acquired

obesity. *Science (Wash. DC).* 237:405.
17. Hiemstra, P.S., E. Langeler, B. Compier, Y. Keepers, P.C.J. Leijh, M.T. van den Barselaar, D. Overbosch, and M.R. Daha. 1989. Complete and partial deficiencies of complement factor D in a Dutch family. *J. Clin. Invest.* 84:1957.
18. Futter, C.E., D. Panton, S. Kestin, and D.J. Flint. 1992. Mechanism of action of cytotoxic antibodies to adipocytes on adipose tissue, liver and food intake in the rat. *Int. J. Obes.* 16:615.