

IN VIVO EFFECTS OF ANTILYMPHOCYTE ANTIBODIES ON IMMUNITY AND AUTOIMMUNITY

WILLIAM E. SEAMAN, NANCY GUTSTEIN & DAVID WOFSY

Department of Medicine, San Francisco VA Medical Center and University of California, San Francisco, USA

Antisera against lymphocytes have been in clinical use for almost two decades as therapy for the rejection of organ transplants (Heyworth M, 1982). The development of monoclonal antibodies (MAb) against lymphocytes has identified new functional subsets of lymphocytes and has permitted their selective elimination in vivo (Ritz, 1985; Levy, 1985; Ortho Multicenter Transplant Study Group, 1985). In vitro, some antilymphocyte MAb alter the function of lymphocytes, by acting either as agonists (Meuer et al., 1984; Ledbetter et al., 1985) or antagonists (Reinherz et al., 1981; Spits et al., 1982) for cell activation. Thus, antilymphocyte MAbs may find use in vitro not only to eliminate lymphocyte subsets but also to activate or inactivate the target cells.

Studies in our laboratory have focused primarily on the in vivo effects of MAb to mouse T lymphocytes, particularly T "helper/inducer" cells. These can be identified by MAb GK1.5, which reacts with the surface antigen, L3T4, the mouse homologue for human CD4 (Dialynas et al., 1983). GK1.5 is a rat IgG2b MAb, an isotype that is particularly efficient in depleting target cells in vivo. We and others have found that depletion of L3T4 cells from normal mice (i) abolishes humoral immunity (Cobbold et al., 1984; Wofsy et al., 1985), (ii) reduces cellular immunity (Cobbold et al., 1984; Woodcock et al., 1986) and (iii) permits the induction of antigen-specific immune tolerance to some antigens (Benjamin & Waldmann, 1986; Gutstein et al., 1986). The rat anti-L3T4 MAb also induces tolerance to itself, so there is no response by the host to the MAb (Gutstein et al., 1986).

In several murine models for autoimmunity, depletion of L3T4⁺ cells prevents and, in some instances, reverses autoimmunity (Waldor et al., 1985; Wofsy & Seaman, 1985; Ranges et al., 1985; Wofsy, 1986; Christadoss & Dauphinee, 1986). We have studied in particular NZB/NZW F₁ (B/W) mice, which spontaneously develop an autoimmune disease with features of human systemic lupus erythematosus (SLE). These features include antibodies to DNA, with consequent immune complex glomerulonephritis,

leading to renal failure and death. In published studies, we used weekly injections of anti-L3T4 MAb to produce sustained depletion of L3T4⁺ cells from B/W mice, from age 4 to 12 months (Wofsy & Seaman, 1985). At age 4 months the onset of treatment, B/W mice had little autoimmunity. Mice depleted of L3T4⁺ cells beginning at this age failed to generate autoantibodies, had little renal disease, and survived significantly longer than untreated controls. Thus, L3T4⁺ cells are required for the development of autoimmunity in this murine model for SLE.

In recent studies, we have demonstrated that depletion of L3T4⁺ cells can also reverse established disease in B/W mice (Wofsy & Seaman, 1987). In these studies, the initiation of treatment was delayed until age 7 months, when all mice had autoantibodies, the majority had significant proteinuria and/or elevated blood urea nitrogen, and 20 % had died. When treatment with anti-L3T4 MAb was begun at this age, the MAb was less efficient in depleting L3T4⁺ cells than in younger mice or in normal mice; more than 8 weeks of weekly treatment with 2mg of anti-L3T4 were required to deplete L3T4⁺ cells by > 90%. Presumably, this reflects the known defect, in both murine and human SLE, in the clearance of antibody-coated cells by the mononuclear phagocyte system (Frank et al., 1979; Shear et al., 1981). In accord with this, blood L3T4⁺ cells were saturated with anti-L3T4 MAb from the onset of treatment.

Although the decline in L3T4⁺ cells was gradual in these mice, treated mice showed a significant difference from controls within 8 weeks with regard to anti-DNA antibodies and there ensued a progressive and significant improvement in blood urea nitrogen and proteinuria. The median survival in treated mice was > 18 months, compared to 8 months in controls ($p < 0.01$). We conclude that L3T4⁺ cells are required not only to initiate autoimmunity in B/W mice but also to sustain it.

Because the SLE-like disease in B/W mice has many similarities to human SLE, these find-

ings suggest that depletion of CD4⁺ cells from patients with SLE might reverse the autoimmune disease. However, such treatment would also render the patients profoundly immunosuppressed, and thus at risk for infections and possibly malignancy. In mice, cessation of therapy with anti-L3T4 MAb is followed by gradual recovery of L3T4⁺ cells, with consequent restoration of normal immune function (except for the possibility of immune tolerance to soluble antigens presented during the period of cell depletion). Recovery of normal immunity, however, requires a period of up to 4 weeks (Seaman, W.E. et al., unpublished observations). Clinically, it would be desirable if immune suppression by anti-L3T4 could be more rapidly reversed. One approach that may permit this is the use of F(ab')₂ anti-L3T4 MAb.

The rationale for the *in vivo* use of F(ab')₂ anti-L3T4 is based on *in vitro* studies with anti-L3T4 in the absence of complement, anti-L3T4 MAb does not destroy L3T4⁺ cells. Anti-L3T4, however, inhibits the *in vitro* response of L3T4⁺ cells to antigen on antigen presenting cells (APC) (Swain et al., 1984). Anti-L3T4 inhibits the binding of L3T4⁺ cells to APC, suggesting a role for L3T4 in cell adhesion. Other studies, however, indicate that anti-L3T4 delivers inhibitory signals for cell activation (Wassmer et al., 1985). By either mechanism, anti-L3T4 has the capability of inhibiting the function of L3T4⁺ cells without depleting them. Similar results have been obtained using anti-CD4 with human cells.

Based on these findings, we reasoned that F(ab')₂ anti-L3T4 might inhibit the function of L3T4⁺ cells *in vivo* without depleting them. L3T4⁺ cells coated with F(ab')₂ anti-L3T4 would not bind to Fc receptors nor fix complement, so that target cells would not be depleted. We first tested the effects of F(ab')₂ anti-L3T4 on the humoral immune response to bovine serum albumin (BSA) in complete Freund's adjuvant (Gutstein & Wofsy, 1986). Mice treated every other day with 1 mg of F(ab')₂ anti-L3T4 had saturation binding of blood L3T4⁺ cells, with no cell depletion. The immune response to BSA was > 95% inhibited throughout the 3 weeks of treatment. Upon sacrifice of the mice at this point, it was found that L3T4⁺ cells in the spleen and lymph nodes were not saturated with F(ab')₂ anti-L3T4, indicating that inhibition of antibody production did not require blockade of all L3T4 molecules.

We next examined whether F(ab')₂ anti-L3T4 could, like intact anti-L3T4, permit the induction of antigen-specific immune tolerance (Gutstein, N.L. et al., unpublished observations). For these studies we used, as an antigen, a rat MAb that does not react with mouse cells (antibody 2C7, rat IgG2a). The response of BALB/c mice to 2C7 is predominantly against idio-type determinants that are not expressed on the anti-L3T4 MAb (rat Ig2b). When 2C7 is given together with intact anti-L3T4, i.e. when L3T4⁺ cells are depleted, the mice develop long-term, antigen-specific tolerance to 2C7. To examine whether treatment with F(ab')₂ anti-L3T4 would do the same, a group of 10 mice was treated with 1 mg of fragments every other day for 18 days. Beginning with the first injection, the mice also received 1 mg of 2C7 weekly. As a control, immunization with 2C7 was not initiated until 3 days after the 18 day period of treatment with F(ab')₂ anti-L3T4. This control group responded normally to 2C7. In contrast, the group injected with 2C7 during the treatment with F(ab')₂ anti-L3T4 remained unresponsive to 2C7 for at least 24 weeks thereafter, even though weekly injections were continued for 12 weeks and were repeated at 18 weeks and 23 weeks.

From these studies, we conclude that F(ab')₂ anti-L3T4 can be used to suppress humoral immunity and induce tolerance (to at least some antigens), without extensive depletion of L3T4⁺ cells. The half-life of F(ab')₂ anti-L3T4 is short; within 48-72 hrs, no antibody can be detected on L3T4 cells. This permits rapid recovery of normal immune function.

We have begun to examine the effects of F(ab')₂ anti-L3T4 in B/W mice. These studies are complicated by the finding that B/W mice more readily produce antibodies to F(ab')₂ anti-L3T4 than do normal mice, which produce no response to 1 mg of fragment every other day. Thus, in B/W mice treated with F(ab')₂ anti-L3T4, there was suppression of autoantibody production, but there was an increase in immune complex glomerulonephritis that correlated with the appearance of host antibodies to the F(ab')₂ anti-L3T4. One approach to circumvent this problem is to give a single injection of intact anti-L3T4 at the onset of therapy. This renders the mice tolerant to the antibody and to its F(ab')₂ fragment (Wofsy, D. et al., unpublished observations). This approach is now being attempted prior to prolonged treatment of B/W mice with F(ab')₂ anti-L3T4.

We have also treated B/W mice with short courses of intact anti-L3T4 with the hope that such treatment might render the mice tolerant to autoantigen, i.e., DNA. This has not proved successful. Short courses of anti-L3T4 retard autoimmunity, but the autoimmune response recovers when treatment is stopped (Wofsy, D. et al., unpublished observations).

Ideally, treatment of autoimmunity by anti-lymphocyte MAbs would be directed uniquely against cells involved in autoreactivity, leaving intact the response to foreign antigens. Treatment of mice with anti-L3T4, or of humans with anti-CD4, does not permit this selectivity. It has, nonetheless, permitted new insights into the regulation of autoimmunity. It also provides a potential means of immune suppression that is more specific in its action than cytotoxic agents.

REFERENCES

- BENJAMIN, R.J. & WALDMANN, H., 1986. Induction of tolerance by monoclonal antibody therapy. *Nature*, *320* :449-451.
- CHRISTADOSS, P. & DAUPHINFE, M.J., 1986. Immunotherapy for myasthenia gravis: a murine model. *J. Immunol.*, *136* :2437-2440.
- COBBOLD, S.P.; JAYASURIYA, A.; NASH, A.; PROSPERO, T.D. & WALDMANN, H., 1984. Therapy with monoclonal antibodies by elimination of T cell subsets in vivo. *Nature*, *312* :548-551.
- DIALYNAS, D.P.; QUAN, Z.S.; WALL, K.A. et al., 1983. Characterization of the murine T cell surface molecule, designated L3T4, identified by monoclonal antibody GK1.5: similarity of L3T4 to the human Leu-3/T4 molecule. *J. Immunol.*, *131* :2445-2451.
- FRANK, M.M.; HAMBURGER, M.I.; LAWLEY, T.J.; KIMBERLY, R.P. & PLOTZ, P.H., 1979. Defective reticuloendothelial system Fc-receptor function in systemic lupus erythematosus. *N. Engl. J. Med.*, *300* :518-523.
- GUTSTEIN, N.L.; SEAMAN, W.E.; SCOTT, J.H. & WOLFSY, D., 1986. Induction of immune tolerance by administration of monoclonal antibody to L3T4. *J. Immunol.*, *137* :1127-1132.
- LEVY, R., 1985. Biologicals for cancer treatment: monoclonal antibodies. *Hospital Practice Nov.*, *15* :67-84.
- GUTSTEIN, N.L. & WOLFSY, D., 1986. Administration of F(ab')₂ fragments of monoclonal antibody to L3T4 inhibits humoral immunity in mice without depleting L3T4⁺ cells. *J. Immunol.*, *137* :3414-3419.
- HEYWORTH, M., 1982. Clinical experience with anti-lymphocyte serum. *Immunol. Rev.*, *65* :79-97.
- LEDBETTER, J.A.; MARTIN, P.J.; SPOONER, C.E. et al., 1985. Antibodies to Tp67 and Tp44 augment and sustain proliferative responses of activated T cells. *J. Immunol.*, *135* :2331-2336.
- MEUER, S.C.; HUSSEY, R.E.; FABBI, M. et al., 1984. An alternative pathway of T-cell activation: a functional role for the 50 kD T11 sheep erythrocyte receptor protein. *Cell*, *36* :897-906.
- Ortho Multicenter Transplant Study Group, 1985. A randomized trial of OKT3 monoclonal antibody for acute rejection of cadaveric renal transplants. *N. Engl. J. Med.*, *313* :337-342.
- RANGES, G.E.; SRIRAM, S. & COOPER, S.M., 1985. Prevention of type II collagen-induced arthritis by in vivo treatment with anti-L3T4. *J. Exp. Med.*, *162* :1105-1110.
- REINHERZ, E.L.; HUSSEY, R.E.; FITZGERALD, K.; SNOW, P.; TERHORST, C. & SCHLOSSMAN, S. F., 1981. Antibody directed at a surface structure inhibits cytolytic but not suppressor function of human T lymphocytes. *Nature*, *294* :168-170.
- RITZ, J., 1985. Use of monoclonal antibodies in autologous and allogeneic bone marrow transplantation. *Clin. Haematol.*, *12* :813-832.
- SHEAR, H.L.; ROUBINIAN, J.R.; GIL, P. & TALAL, N., 1981. Clearance of sensitized erythrocytes in NZB/NXW mice. Effects of castration and sex hormone treatment. *Eur. J. Immunol.*, *11* :776-780.
- SPITS, H.; BORST, J.; TERHORST, C. & DeVRIES, J.E., 1982. The role of T cell differentiation markers in antigen-specific and lectin-dependent cellular cytotoxicity mediated by T8⁺ and T4⁺ human cytotoxic T cell clones directed at class I and class II MHC antigens. *J. Immunol.*, *129* :1563-1569.
- SWAIN, S.L.; DIALYNAS, D.P.; FITCH, F.W. & ENGLISH, M., 1984. Monoclonal antibody to L3T4 blocks the function of T cells specific for class II major histocompatibility complex antigen. *J. Immunol.*, *132* :1118-1123.
- WALDOR, M.K.; SRIRAM, S.; HARDY, R. et al., 1985. Reversal of experimental allergic encephalomyelitis with monoclonal antibody to a T-cell subset marker. *Science* *227* :415-417.
- WASSMER, P.; CHAN, C.; LÖGDBERG, L.; SHEVACH, E.M., 1985. Role of the L3T4-antigen in T cell activation. II. Inhibition of T cell activation by monoclonal anti-L3T4 antibodies in the absence of accessory cells. *J. Immunol.*, *135* :2237-2242.
- WOLFSY, D.; SEAMAN, W.E., 1985. Successful treatment of autoimmunity in NZB/NZW mice with monoclonal antibody to L3T4. *J. Exp. Med.*, *161* :378-391.
- WOLFSY, D.; MAYES, D.C.; WOODCOCK, J.; SEAMAN, W.E., 1985. Inhibition of humoral immunity in vivo by monoclonal antibody to L3T4: studies with soluble antigens in intact mice. *J. Immunol.*, *135* :1698-1701.
- WOLFSY, D. & SEAMAN, W.E., 1987. Reversal of advanced murine lupus in NZB/NZW F₁ mice by treatment with monoclonal antibody to L3T4. *J. Immunol.*, In press.
- WOODCOCK, J.; WOLFSY, D.; ERIKSSON, E.; SCOTT, J.; SEAMAN, W.E., 1986. Rejection of skin grafts and generation of cytotoxic T cells by mice depleted of L3T4⁺ cells. *Transplantation*, *42* :636-642.