Reviews in Medicine

Clinical immunology

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Introduction

The breadth of coverage of this successful review series, which commenced in January 1990, presents particular problems for the clinical immunologist, whose discipline has consequences for so many medical specialties. A therapeutic approach has been adopted in an attempt not only to give a theme to this review but also to minimize overlap with reviews from earlier in the series. Advances in the field of autoimmunity will be addressed in the latter part of the review with discussion of their impact on patient management. Chronic fatigue syndrome has appeared in several of the reviews in this series already, and consequently only certain areas will be highlighted.

Monoclonal antibody therapy

The therapeutic possibilities of selectively attacking cell populations implicated in the pathogenesis of a variety of immunologically mediated diseases have always been attractive.

In vasculitis and autoimmune diseases

Antibodies to surface markers on lymphocytes, particularly mature T cells (anti-CD3) have an established role in the treatment of clinical allograft rejection.¹ However, the use of mouse monoclonal antibodies in man often leads to the generation of human anti-mouse antibodies. In a study of 8 patients with multiple sclerosis, daily infusions of either mouse anti-CD2 (pan T cell marker) or mouse anti-CD4 (T helper phenotype) led to the generation of significant anti-idiotypic activity in 2 of 8 patients despite *in vitro* evidence of immunomodulation while undergoing the infusions.² Therefore the long term or repeated use of such therapeutic antibodies can only be possible if the agents are rendered less immunogenic. Consequently, a brief report in the *New England Journal*

of Medicine describing the use of a genetically engineered 'humanized' form of rat antibody in a patient with systemic vasculitis was of particular interest.³

In fact two monoclonals were given to establish a long lasting remission in a patient with a previously intractable systemic vasculitis. The humanized antibody was campath-1H which recognizes the CDw 52 cluster (expressed by many leucocytes), while the other was a rat antibody to the CD4 molecule on T helper cells. The combination of these 2 antibodies significantly improved the disease status yet no serological response to human or rat immunoglobulins was identified. This form of combination therapy will have significant implications for the management of human autoimmune diseases.

A German group has published an elegant open study on 10 patients with severe intractable rheumatoid arthritis who received 7 daily infusions of a mouse anti-CD4 monoclonal antibody.⁴ Studies of the kinetics of the peripheral mononuclear cell response demonstrated a drastic depletion of CD4 + cells to as low as 25 cell/ μ l 1 hour after the first infusion, from mean starting CD4 + counts of $820 \pm 410/\mu$ l. The CD4 + cell numbers at 24 hours after the infusion had not reached initial levels and after the full 7 days treatment a significant reduction in CD4 + cells remained and persisted for 3–4 weeks. Lymphocyte transformation assays showed reduced reactivity in 5 of the 9 patients who completed the course of infusions, whereas, in contrast, 4 individuals exhibited an unexpected increase in the T cell responses to the mitogen PHA (phytohaemagglutinin) and common antigens such as tetanus and diphtheria toxoids and tuberculin (PPD). Clinical improvement was evident by the end of the 7 day treatment schedule as measured by the number of swollen joints, Ritchie index and functional ability. Significant falls in the erythrocyte sedimentation rate and C-reactive protein were apparent. This improvement lasted 6 months in 2 patients after 7 daily infusions and in a further 2 subjects, after 2 cycles of 7 days therapy. Adverse reactions included urticaria in 2 patients which led

to the withdrawal from the protocol in one of them. Two additional patients experienced chills with fever suggesting a lymphokine release syndrome. Serum anti-mouse antibodies were detected in the patients; however, the levels were considered to be quite low (<1.7 mg/l) and repeat cycles of treatment were given to 4 patients without significant reactions. No episodes of infection were noted in this patient group during observation periods up to one year.

In septic shock

Endotoxin is the lipopolysaccharide component of Gram-negative bacterial cell walls and when released from these bacteria into the circulation it can produce the adverse systemic reactions we associate with septic shock.⁵ A human IgM monoclonal antibody (HA-1A) that binds specifically to the lipid A domain of endotoxin has been produced and its value in patients with sepsis and Gram-negative infection assessed.⁶ Five hundred and forty-three patients with presumed Gram-negative infection were enrolled in a randomized double-blind trial, to be given either a single infusion of 100 mg dose of HA-1A or a placebo, in addition to the conventional therapy, for example antibiotics, fluid replacement, cardio-respiratory support; 200 patients had subsequently proven Gram-negative bacteraemia with a significant reduction in deaths in the HA-1A treated group. In fact, there were 49% fatalities in the placebo group and 30% fatalities in the recipients of HA-1A ($P = 0.0014$). Improved survival was demonstrable both in the severely ill and the less severely ill patients. No benefit was noted in patients with sepsis who subsequently proved not to have Gram-negative bacteraemia and no anti-HA-1A antibodies were detectable. Use of this IgM monoclonal was not associated with adverse reactions and it is particularly valuable because it reacts with the endotoxin of many Gram-negative bacteria. The cost of this reagent will be considerable but when compared to the savings on intensive care therapy, it surely will gain a place in the treatment of this often fatal condition.

The cytokine, tumour necrosis factor (TNF) is implicated in many disease states such as sepsis, cancer and burns. Phase one studies of a murine monoclonal antibody to recombinant human TNF has now been reported in patients with septic shock.⁷ This therapy was found to be safe and without side effects. Further studies are in progress and their results are keenly awaited. Interestingly a role for tumour necrosis factor in familial mediterranean fever has recently been suggested.⁸

Therefore, early clinical studies with monoclonal antibodies show great potential and the possibility

of using humanized xenogenic antibodies for such therapies should hold even greater promise.

Intravenous immunoglobulin therapy (IVIg)

IVIg infusions have become popular in the last decade either as replacement therapy for antibody deficiency states, for example hypogammaglobulinaemia, or in the treatment of various disorders characterized by vasculitis/autoimmunity.

For antibody deficiency states the value of IVIg every 3–4 weeks is accepted and has largely replaced intramuscular preparations due to greater compliance, painless infusions and the ability to give larger doses. The prevention of infections even in those patients with severe chest disease is notable.^{9,10} The current generation of IVIg preparations with its low incidence of adverse reactions, has facilitated the establishment of home therapy IVIg where patients infuse safely in their own homes.¹¹

In acute Kawasaki syndrome IVIg is given to prevent coronary-artery lesions¹² and a single high dose infusion of IVIg appears superior to 4 smaller doses of IVIg in preventing such lesions.¹³

How does IVIg mediate its therapeutic effects? The role of anti-idiotypic antibodies will be discussed in the autoantibody section under anti-idiotypic. Another possible mechanism may be the enhancement of antibacterial activities of phagocytic cells, e.g. neutrophils, consequent upon stimulation of the respiratory burst and promotion of bacterial killing.¹⁴ It is, of course, conceivable that the undesirable reactions (chills, fever, etc.) observed during IVIg infusions in hypogammaglobulinaemic patients, which occur particularly when they have infection, may be related to phagocytic cell activation. IVIg also appears to downregulate monocyte IgG Fc receptors (FCRI and FCRII) which may impair their ability to phagocytoses.¹⁵

C1-inhibitor therapy

Patients with inherited or acquired C1-inhibitor deficiency manifest episodes of angioedema and should these involve the pharynx or larynx then the outcome can be fatal. Until recently tranexamic acid and danazol for prophylaxis and infusions of fresh frozen plasma for severe episodes involving the larynx or internal organs have been the therapeutic options. A concentrate of purified C1-inhibitor is now available (C1 anactivator, P, Behringwerke AG) and appears satisfactory for hereditary and acquired C1-inhibitor deficiency

both as prophylaxis and in acute severe episodes.¹⁶ The price is considerable and this may limit its use to the more problematical cases.

Interferon therapy

The haematology review in this series discussed the use of interferon alpha in clinical myeloid leukaemia¹⁷ and in the gastroenterology review the value of interferon alpha in chronic hepatitis B infection was enumerated.¹⁸ A licence has recently been granted for the use of interferon alpha in the treatment of chronic active hepatitis B. Currently 2 types of interferon alpha are available, namely a recombinant version or the natural human product extracted from human lymphoblastoid cell cultures. The latter contains at least 22 naturally occurring alpha subtypes. Serum neutralizing antibodies occur infrequently with the natural product whereas with the recombinant preparation a significant number develop such antibodies, which will influence efficacy and may be involved in the generation of adverse reactions.¹⁹

The addition of interferon gamma to pentavalent antimony in the treatment of refractory or severe visceral leishmaniasis (kala-azar) is an interesting development. In the cutaneous and mucocutaneous forms of leishmaniasis, the parasite remains localized but in the visceral form of the disease, growth of the parasite continues within the monocyte-macrophage system, resulting in fever, weight loss and hepatosplenomegaly. The great majority of patients with kala-azar respond to pentavalent antimony; however, some have only a temporary response and others require several courses of treatment to achieve a satisfactory outcome. A few patients remain refractory even to repeated courses and the addition of interferon gamma injections in combination with pentavalent antimony seems to herald promise in this problematical group.²⁰ However, because this is predominantly a Third World disease its availability is likely to be limited.

Cytotoxic regimes for vasculitic diseases

'To pulse or not to pulse', was the title of an excellent editorial in the *American Journal of Medicine*.²¹ Cyclophosphamide is an effective treatment in certain vasculitic diseases but when used orally (2 mg/kg) it has a low therapeutic/toxic profile. Attempts to reduce the morbidity associated with oral administration has led to the development of protocols utilizing intermittent therapy (pulse therapy) with larger doses of the drug. The use of pulse cyclophosphamide has proved a major improvement in the treatment of

lupus nephritis with sustained efficacy and decreased toxicity.^{22,23} The success in lupus nephritis has led to a tendency to assume that pulse cyclophosphamide will be comparable or superior to long term daily dosage schedules in other vasculitides. A report by Hoffman *et al.*,²⁴ in the same issue as the editorial, used high dose intravenous pulse cyclophosphamide with oral steroids in 14 patients with Wegener's granulomatosis and noted initial benefit in almost all patients in the study. However, in the majority of cases, improvement was not sustained. This contrasts with the lower relapse rates reported with low dose oral cyclophosphamide treatment protocols.²⁵ This led the authors to conclude that daily low dose oral cyclophosphamide and steroids remain the treatment of choice for Wegener's granulomatosis. The implications for other non-systemic lupus erythematosus (SLE) vasculitides, for example polyarteritis, are considerable and appropriate clinical studies must be completed to establish the optimum regimes for cyclophosphamide in the various different vasculitides.

Therapy for individuals with human immunodeficiency virus infection

Recognition of the diverse immunological defects associated with infection by the human immunodeficiency virus type 1 (HIV) has led to the development of novel approaches to its treatment. Antiviral agents directed against HIV itself, such as zidovudine (AZT or azidothymidine), a potent inhibitor of HIV replication, remain the most effective treatments. Unfortunately, clinical predictors of disease progression tend to be evident relatively late in the course of HIV infections, when significant impairment of the immune system has already occurred. Consequently, laboratory markers of disease progression in asymptomatic HIV positive individuals are being assessed. CD4 lymphocyte counts, serum HIV-1 p24 core antigen, β 2-microglobulin and neopterin have all been identified as having predictive value for the progression to AIDS.²⁶⁻³² The combination of CD4 counts and p24 antigenaemia in predicting progression to AIDS has recently been addressed.³³ The presence of p24 antigenaemia is associated with a more rapid decline in the CD4 + lymphocyte population and in conjunction with CD4 counts is useful in identifying those individuals at significantly greater risk of disease progression over a 4 year period.

The results of an American randomized double blind trial of zidovudine to assess the value of treating asymptomatic HIV infected adults with CD4 cell counts < 500 mm³ at entry to the study have been published.³⁴ The study treatment groups included AZT 500 mg/day, 1500 mg/day, or place-

bo. AZT 1500 mg/day was associated with severe haematological toxicity, but with 500 mg AZT only nausea was a problem. After a mean follow up of 55 weeks (range 19–107) 33 subjects assigned to placebo had AIDS, compared with 11 on 500 mg AZT (confidence interval 1.4–5.6) and 14 of those receiving 1500 mg AZT (confidence interval 10–13.5). Whether such treatment will improve the survival for persons with HIV and whether health care services can afford the cost remains to be determined.

Inosine pranobex, a so-called immuno-modulant has also been tried in HIV infection³⁵ in a randomized placebo controlled trial of 866 HIV infected people in Sweden and Denmark. Significantly fewer individuals progressed to acquired immunodeficiency syndrome (AIDS) during the 24 week study period in the group who received inosine pranobex (3 g/day), than those who received placebo (2 versus 17). This finding is interesting and will need validation. Certain features of the study require highlighting such as the alteration of the primary end point designated before the study began, to a new end point when the data was analysed. Also no significant difference in the decreases in CD4+ cell counts was observed between the groups or the level of p24 antigenaemia. For further discussion of this provocative report the reader is referred to an editorial entitled 'Inosine pranobex – is a single positive trial enough?'³⁶

A review of combination anti-retroviral therapy is beyond the scope of this immunological review but the reader is referred to the proceedings of a recent symposium on this topic.³⁷

Although mouth ulceration in patients with AIDS is common, some HIV positive individuals develop severe recurrent oral ulcers unrelated to malignancy or fungal infection. Histologically these ulcers resemble those seen in Behçet's disease and the beneficial effects of thalidomide in Behçet's ulceration led to its use in AIDS with considerable success.³⁸ Thalidomide in childbearing women is contra-indicated and the patients must be monitored with regular conduction studies, to prevent the development of an irreversible painful peripheral neuropathy.

Strategies for the treatment of allergy

Various strategies are being developed for immunological intervention in IgE-mediated hypersensitivity reactions. The limitations to allergen immunotherapy include problems with allergen identification particularly with food and drug sensitization. The possibility of severe adverse reactions further restrict its use in the UK.^{39,40} A peptide vaccine to provide a new form of immuno-

therapy in patients with IgE mediated reactions seems much closer following a study by Stanworth *et al.*⁴¹ They immunized rabbits with a decapeptide sequence from within the CH4 domain of human IgE conjugated with carrier molecules such as keyhole limpet haemocyanin (KLH) or tuberculin in Freund's adjuvant to obtain polyclonal anti-peptide antibodies. These antibodies were able to inhibit histamine release from rat mast cells both *in vivo* and *in vitro*. Immunization of rats, previously sensitized to ovalbumin with the peptide, reduced the IgE antibody formation and the serum histamine concentration. Furthermore it abolished the systemic anaphylactic reaction in response to allergen challenge. Clinical trials will be needed to examine the value of this new immunotherapeutic technique which should have exciting clinical prospects.

A further strategy to reduce immediate hypersensitivity responses might be to functionally inactivate allergen reactive T cell clones. Staphylococcal enterotoxins are capable of stimulating powerful polyclonal proliferative responses and to induce non-responsiveness of T lymphocytes expressing the appropriate TCR V β gene products. O'Hehir *et al.*⁴² have identified T cell clones involved in the human responses to house dust mite (*Dermatophagoides* Spp) that express either V β 3 or V β 6 gene products. Pre-treatment of clonal V β 3 T cells with *Staphylococcus aureus* enterotoxins A or C1 rendered them non-responsive to immunogenic challenge with their natural ligand, namely house dust mite, while retaining responsiveness to exogenous interleukin 2 (IL2). Similarly, exposure to the V β 6 + dust mite reactive T cells to the staphylococcal enterotoxin of appropriate specificity, SEE, induced specific anergy. Toxicity of staphylococcal enterotoxins can be removed by chemical modification and therefore their ability to functionally inactivate subpopulations of T cells expressing antigen specific receptors with shared characteristics may be of value in the treatment of allergic disease.

Anti-idiotypic antibodies are involved in the regulation of a number of immune responses including IgE production. In atopic patients, increased IgE synthesis may be related to defective production of regulatory anti-idiotypic antibodies. Hebert *et al.*⁴³ have reported higher levels of anti-idiotypic antibodies to monoclonal antibodies directed against rye grass pollens in normal individuals compared to untreated atopic patients. Specific immunotherapy with grass pollen extracts increased the anti-idiotypic antibody levels to those of normal volunteers. No details of the clinical responses to the specific immunotherapy were supplied.

In summary, the prospects for allergy therapy seem good and the next decade should prove

fruitful for at least one of these avenues of therapeutic intervention.

Autoimmunity

Alcoholic liver disease

Autoimmune mechanisms may be implicated in the generation of alcoholic liver disease. The observation that only 15–20% of alcoholics will develop liver disease has not been fully explained. A Canadian group⁴⁴ compared the autoantibody profiles in alcoholics with and without liver disease and in a group of patients with chronic active hepatitis (CAH). They noted a higher incidence of antibodies to nuclei, single and double-stranded DNA and anti-lymphocytic antibodies in the alcoholics with liver disease than those without. Furthermore, the autoantibody profile in the alcoholics with liver disease was not dissimilar to the CAH group, although the incidence of antinuclear antibodies was less. Unfortunately, the alcoholic no liver damage group were all male, whereas 20% of the alcoholic liver damage subjects were female and ethical restrictions prevented liver biopsies in the alcoholic no liver damage group so that a 'clean' liver was defined by liver function tests alone.

Autoimmune polyendocrinopathy

Circulating organ specific autoantibodies are serological markers for the destruction or impairment of the relevant endocrine tissue cells in this condition. Bottazzo's group have reported the presence of organ specific cardiac antibodies in 17% of patients with autoimmune polyendocrinopathy compared to 1% of those with autoimmunity affecting only one gland and 3.5% of normal subjects.⁴⁵ In addition, the prevalence of systemic hypertension in the polyendocrinopathy group was higher in those with cardiac autoantibodies than in those without (18% vs 3%). Prospective studies are now required to confirm whether these cardiac organ specific antibodies characterize a subgroup of patients with systemic hypertension.

IgA polyspecific autoantibodies in IgA nephropathy

The specificity of circulating and kidney bound IgA in patients with IgA nephropathy has recently been investigated⁴⁶ and follows the demonstration of serum antibodies to bovine serum albumin,⁴⁷ and gliadin,⁴⁸ alpha galactosyl⁴⁹ and autoantibodies to collagen type IV⁵⁰ and rheumatoid factors⁵¹ in these patients. Evidence for the presence of viral antigens, for example cytomegalovirus, in the mesangium of patients with IgA nephropathy has also been presented but not substantiated.⁵² However,

the study reviewed here suggests that these findings may result from the generation of polyspecific IgA antibodies and recommends considerable caution should be exercised when drawing conclusions from the finding of apparently monospecific IgA antibodies in this condition.

Monoclonal autoantibodies in polyneuropathy

Monoclonal antibodies directed against myelin antigens are frequently detected in patients with polyneuropathy and can result in distal weakness, tremor, ataxia and loss of proprioception. Colocalization along the myelin sheaths of peripheral nerve fibres of monoclonal proteins and C1q, C3d and C5 has now been identified by immunostaining.⁵³ A correlation between the numbers of fibres with abnormally spaced myelin and the extent of deposition of terminal complement complex suggests that the terminal complex contributes to the myelin damage itself. Previously it was considered that the IgM monoclonal protein physically impaired the myelin sheaths and the extent of complement involvement was unclear.

Autoantibodies and autoantigens in type 1 diabetes mellitus

Accurate identification of the individuals who will develop insulin dependent diabetes mellitus (IDDM) is paramount if therapeutic strategies to preserve the maximum beta-cell mass are to be successful. Studies on the cohort recruited by the Bart's-Windsor prospective family study of diabetes⁵⁴ has allowed the sensitivity and predictive value of islet cell antibodies (ICA) measured by immunofluorescence in Juvenile Diabetes Foundation (JDF) units to be ascertained over an extended follow-up period. In all 5 relatives with peak ICA levels above 80 JDF units, IDDM developed within a 7-year follow-up. Survival without IDDM at 10 years was 27% among relatives with peak ICA levels of 20–80 JDF units, and 82% for peak ICA levels of 4–20 JDF units. The predictive values for IDDM development within 10 years ranged from 40% (threshold 4 JDF units) to 100% (80 JDF units) and the sensitivity from 31% (80 JDF units) to 88% (4 JDF units). It is unlikely that any single centre could achieve a larger study group, but with the standardization of ICA antibody testing in JDF units, pooling of data from comparable studies will now be possible.

The search for the autoantigens relevant to the pathogenesis of IDDM continues. A 64 kD antigen recognized by serum autoantibodies associated with IDDM has been pinpointed as glutamic acid decarboxylase (GAD) which is the biosynthesizing enzyme of the neurotransmitter GABA (gamma amino butyric acid).⁵⁵ A major autoantigen recog-

nized by T cells isolated from patients with recent onset IDDM has also been identified.⁵⁶ It appears to be a 38 kD insulin-secretory granule protein present in the islet β cells. The cataloguing of such autoantigens, particularly those recognized by T cells, may lead to specific immunotherapy for diabetes, through the generation of peptide analogues of the antigenic epitopes or the T cell receptor sequences involved in their recognition.⁵⁷

Autoantibodies in stiff-man syndrome

Stiff-man syndrome is a rare disorder characterized by progressive rigidity and painful spasms of the skeletal muscles. Auto-antibodies to glutamic acid decarboxylase (GAD) were reported in a patient with stiff-man syndrome, epilepsy and Type 1 diabetes mellitus.⁵⁸ GAD is an enzyme selectively concentrated in neurones secreting the neurotransmitter gamma-aminobutyric acid (GABA) and in pancreatic beta cells. The same group systematically studied a large group of these patients and observed autoantibodies to GABA-ergic neurones in 20 of 33 patients.⁵⁹ GAD featured as the principal autoantigen. In those 20 positive patients there was striking association with organ specific autoimmune diseases, especially insulin dependent diabetes mellitus. These findings strongly support the hypothesis that stiff-man syndrome is an autoimmune disease.

Anti-neutrophil cytoplasmic antibodies

The identification of autoantibodies to neutrophil cytoplasmic antigens (ANCA) in the sera of patients with an active systemic vasculitis, such as Wegener's granulomatosis (WG) and polyarteritis nodosa (PAN), has provided a means of rapid diagnosis in many patients.⁶⁰ Hitherto, no serological test was available to differentiate the disease spectrum of systemic vasculitis especially when the clinical presentation was atypical. The classical anti-neutrophil cytoplasmic antibody (cANCA), detected by indirect immunofluorescence on normal human neutrophils, is positive in the majority of cases of WG and PAN at presentation.⁶¹ A longitudinal study has demonstrated the value of titration of cANCA by immunofluorescence in the monitoring of disease activity in WG and PAN.⁶² Titres of cANCA were markedly raised both at presentation and at vasculitic relapse in 10 patients, but intercurrent episodes such as infection or pulmonary embolism were not associated with increases in the cANCA titres. In contrast, increases in serum C-reactive protein (CRP) could not distinguish non-vasculitic illness from vasculitic relapse. Previously only CRP had been correlated with increases in disease activity;^{63,64} however, C-reactive protein remains a better mea-

sure of recovery as its half-life is 8 hours compared to that of IgG whose half-life is 3 weeks.

The relationship between the immunofluorescence patterns of cANCA and pANCA (perinuclear pattern) and the results of ELISA and solid-phase radioimmunoassay remain unclear.⁶⁵

The classical cANCA immunofluorescence pattern is rarely found in vasculitis other than due to WG and PAN. However, other distinct non-cANCA patterns have been noted in a variety of conditions, such as rheumatoid arthritis, Felty's syndrome, Churg-Strauss syndrome and microscopic polyarteritis.

Furthermore the classic (isotype) antibody to neutrophil cytoplasmic antigens may also be relevant to clinical disease expression. Isolated IgM ANCA is reported to occur in patients with severe pulmonary haemorrhage and nephritis⁶⁶ and IgA with IgG ANCA are found in adult Henoch-Schönlein purpura.⁶⁷ The ANCA IgG subclass distribution by radioimmunoassay in patients with systemic vasculitis (including PAN and WG) shows a relative enrichment of IgG 3 and depletion of IgG 2. When active and remission sera were compared, IgG 3 ANCA levels had fallen and IgG2 ANCA levels increased with remission.⁶⁸

A pathogenetic role for ANCA in PAN and WG is suggested by the correlations between ANCA titre and disease expression. One might question the ability of these antibodies to penetrate viable cells to produce immunological effects. However, recent studies have unequivocally demonstrated that antibody can enter viable human lymphocytes,^{69,70} hence alter cell antigens and thus play a direct role in the immunopathological process.

The presence of anti-idiotypes against ANCA in normal immunoglobulin preparations used for intravenous infusion (IVIg) suggest that such IVIg infusion may have a therapeutic role in systemic vasculitis. Such anti-idiotypes have also been demonstrated in the sera of patients with vasculitis when they are in remission.⁷¹ A recent publication confirms the beneficial effect of IVIg infusions in patients with systemic vasculitis who have active disease despite conventional therapies.⁷² The role of anti-endothelial cell antibodies (AECA) in vasculitis remains debatable but a combined role of ANCA and AECA in the generation of the vasculitic process remains a strong possibility.⁷³ AECA do not appear to be mere epiphenomena consequent to inflammatory vascular injury as they are present in some vasculitic conditions such as WG and PAN yet not in essential mixed cryoglobulinaemia.⁷⁴

Chronic fatigue syndrome

This disabling condition of unknown aetiology is characterized by extreme fatigue exacerbated by

minimal physical activity, reduced powers of concentration and short term memory loss. The search continues for aetiological factors and worthwhile approaches to therapy. Wider use of the term chronic fatigue syndrome (CFS) by doctors and the lay public rather than myalgic encephalomyelitis (ME) or post-viral fatigue syndrome, with their implications concerning unproven assumptions about aetiology, would be a valuable stride forward. Considerable interest in CFS continues and this journal alone carried both a review⁷⁵ and an original article⁷⁶ on CFS in 1990. In addition, an article earlier in this series on 'Infection and Infectious Disease'⁷⁷ paid considerable attention to the condition. Since the latter, 2 contradicting articles on the beneficial effects of intravenous immunoglobulin therapy (IVIg) in CFS appeared in the same issue of the *American Journal of Medicine*. A major difference between the studies was the dose of IVIg, namely 1 mg/kg in the American study, showing no benefit,⁷⁸ whereas 2 mg/kg was used in the Australian study⁷⁹ which showed benefit in 43% of individuals. The American study recruited predominantly female patients from rheumatologists and infectious disease specialists, used a published working case definition for CFS⁸⁰ and has a 20% incidence of adverse reactions in both the IVIg and the placebo (1% albumin solution) groups. In contrast, the Australian study had a major psychiatric input into the assessments, recruited virtually equal groups of male and female patients and all patients had chronic persisting symptoms rather than a relapsing remitting course. In fact, the mean duration of symptoms was 47 months in the Australian study. Such major differences between the studies must leave the question about the beneficial effects of IVIg unanswered.

The rationale behind the use of IVIg in both studies was the concept of an immunoregulatory defect in CFS. The American study concentrated on immunoglobulin subclass deficiencies whereas the Australian study reported abnormal cell mediated immunity in 40 of 49 patients with CFS based on reduced delayed type hypersensitivity skin tests and/or T cell lymphopenia. These immunological abnormalities may well be a consequence of the chronic illness rather than primary defects.

Subtle changes in natural killer cell (NK) phenotypes are reported in CFS, in particular an increase in CD56^{bright+} cells which comprise less than 5% of the entire NK population in normal individuals.⁸¹ However, changes in function relating to altered phenotype expression can only

remain the object of speculation, but would be compatible with persisting viral infection. Continuing the theme of possible viral infection, enteroviral RNA sequences have been detected in the muscle of some patients with CFS by the polymerase chain reaction.⁸² In the 60 patients with CFS, 53% were positive for enteroviral RNA sequences in muscle after polymerase chain reaction amplification and hybridization with the probe EP2 compared to 15% of 41 controls. Persistent enteroviral infection may therefore occur in some patients with CFS and may indeed have an aetiological role. However, the findings of enteroviral sequences did not correlate with duration of disease, age or raised titres of antibodies to coxsackie viruses B1-5. Furthermore a study looking directly at serological tests available for coxsackie B virus antibodies concluded that they were unhelpful in the diagnosis of CFS and simply reflected the background in the population.⁸³

A study which has generated considerable interest in the CFS patient population leading to demands for magnesium injections appeared in the *Lancet* this year.⁸⁴ The study reported lower red cell magnesium concentrations in CFS patients than in healthy controls, and in a trial comparing 6 weekly intramuscular magnesium sulphate and placebo injections describe an improvement in 12 of 15 CFS magnesium treated patients compared to 3 of 17 CFS placebo treated patients. Only 0.3% of total body magnesium concentration is present in plasma and therefore measurement of erythrocyte magnesium to reflect the intracellular action would seem a reasonable approach. However, analytical imprecision appears to reduce the usefulness of this approach, such that plasma magnesium remains the most useful indicator of magnesium status.⁸⁵ In particular red cell magnesium content is dependent on the age of the erythrocyte, being highest in newly produced cells, and correlates poorly with plasma levels.⁸⁶ It is not clear from the *Lancet* paper why the magnesium levels in the case control study and those in the double-blind trial were performed in laboratories 'under slightly different conditions' such that according to the authors they are not comparable. If we accept the findings of this study then we must decide whether they are causal or the product of CFS itself. Certainly magnesium levels can affect the energy status; however, stress, anxiety and nervousness can themselves induce hypomagnesaemia.^{87,88} We must look forward to further studies to help resolve these issues in CFS.

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