Clinical review

Recent advances

Immunology

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Immunology is one of the most recent subjects to have emerged as a separate discipline in biomedical science. Therefore, many clinicians currently practising will have received little or no basic or clinical training in this subject, while those who have will find that much has changed in the past five to 10 years. Access to this important aspect of medicine is hampered by the profligate use of acronyms and jargon, but some attempt has been made to bring order where chaos threatened. The CD (cluster of differentiation) system defines cell surface molecules and now stretches to CD161. The interleukin system of cytokine nomenclature (now up to interleukin 18) has rid the literature of BCGF (B cell growth factor) and other acronyms. The genetic age has brought with it a new nomenclature for the HLA system, which may be difficult for clinicians who are required to adapt from the old one but will be beneficial to new generations of clinicians. The reality is that immunology can no longer be avoided. Its relevance spans from pregnancy to senescence and from vaccination to cancer, a fact recognised by the 10 Nobel prizes for medicine or physiology awarded for key immunological advances, most recently to Zinkernagel and Doherty for their work on the specificity of cell mediated immune defence.

Recent advances

The cellular receptor used by HIV in fusing with target cells has been identified. The natural ligand for the receptor is capable of blocking HIV transmission in vitro, implying a new treatment strategy

Motor vehicle pollution is implicated in the pathogenesis of asthma. Genetic studies link the disease with the gene for interleukin 4, a cytokine known to enhance IgE production

The subdivision of CD4, and now CD8, T lymphocytes into groupings on the basis of cytokine production is promoting a greater understanding of the role of these cells in allergy, autoimmunity, and infection

Immune regulation may depend heavily on killing activated T lymphocytes at the end of an inflammatory episode through the Fas and Fas ligand system. Transplant immunologists are looking to Fas as a way of protecting foreign grafts

The crystallisation of a complex of T cell receptor, peptide antigen, and molecule of the major histocompatibility complex has provided the best insight yet of how these molecules interact to achieve T cell activation

Methods

We have chosen what we consider to be some of the most exciting recent discoveries in immunology, focusing on subjects in which the frontiers of basic immunological research are shaping up to give rise to clinical applications in the next decade.

Mechanisms of resistance to HIV infection

HIV attacks CD4 T lymphocytes and macrophages and dendritic cells (fig 1). Until recently, the CD4 molecule was believed to be the only receptor that HIV uses to enter lymphocytes, but not all of the experimental data supported this. For example, although 10^{10} HIV virions are produced per day, a minority of CD4 T lymphocytes from patients infected with HIV contain the virus and some lymphocytes are resistant to infection. This resistance to HIV infection seems to be associated with the activity of a family of attractant inflammatory peptides (called the CC chemokines), which contribute to cell wall adhesion and tissue migration and are produced by CD8 T lymphocytes.[2] During initial infection HIV enters the cell through the CC-CKR-5 receptor (CC-chemokine receptor)[3] (fig 2). The CC-CKR-5 receptor is activated by the chemokines RANTES (which stands for regulated by activation, normal T cell expressed and secreted), MIP-1α, and MIP-1β (macrophage inflammatory protein 1α and 1β). (Unfortunately, chemokine nomenclature has not yet been simplified and relies on acronyms.) Once a person is infected, however, the CXCR-4 receptor, another chemokine receptor, is used by HIV to enter cells and spread from one CD4 T lymphocyte to another.[4] People who are apparently uninfected despite repeated exposure have allotypic variants of the CC-CKR-5 receptor.

Clearly, if entry of the virus through chemokine receptors into CD4 T lymphocytes, macrophages, and dendritic cells is preventable, then people could be protected. Furthermore, prevention of viral spread in infected patients could maintain the virus at a low concentration, allowing the immune system to eliminate it.

Great strides have been made in developing a vaccine against HIV. Protective mucosal immunity has been elicited by targeted iliac lymph node immunisation with a subunit simian immunodeficiency virus envelope and core vaccine in macaques.[5]

Interaction of genes and environment in allergy

During the past 20-30 years allergy has become increasingly common.[6] Whether this is due to changes to the environment or to genetic factors is unclear. One opportunity to study this interplay has been provided by the reunification of Germany. The prevalence of asthma and allergy was studied in East and West Germany, where over 7500 children aged between 9 and 11 years old from Leipzig and Halle in former East Germany and Munich in former West Germany were compared. The children underwent cold air challenge to assess bronchial hyperreactivity and diagnostic allergy skin prick tests. Allergy, defined as a positive result on skin prick testing with one or more common allergens, was much more prevalent (37%) in the West German children than in their East German counterparts (18%). The prevalence of asthma and hay fever was significantly higher in West Germany (5.9% and 8.6%) than in East Germany (3.9% and 2.7%). Interestingly, bronchitis, cough, and nasal symptoms in the autumn and winter were most prevalent in Leipzig and Halle.[7] In another study of patients with respiratory disease from former East Germany and from Hungary more than 80% of those studied had pre-existing pulmonary damage, such as healed tuberculosis, chronic bronchitis, or emphysema.[8] From these studies, either something in the East German environment, possibly increased infection, protects against allergy—as suggested by Holt[9]—or something in the West German environment, such as motor vehicle pollution, promotes allergy.

The positive association between allergy and motor vehicle use has been investigated at University College Los Angeles. Extracts of polyaromatic hydrocarbons from diesel exhaust fumes acted as mucosal adjuvants which selectively enhanced IgE production in response to common inhaled allergens. These effects were specific to IgE, having no affect on IgG, IgA, or IgM production. These data suggest that diesel exhaust may play an important part in the increased incidence of allergic airway disease.[10]

Genetic factors should not, however, be ignored as allergy is known to run in families. In a study of 170 people from 11 Amish families, linkage was found between raised IgE concentrations and markers on chromosome 5q31.1 (especially with the gene for interleukin 4, a cytokine linked with allergy).[11] Over the next few years all of the genes that control allergy and asthma are likely to be defined.

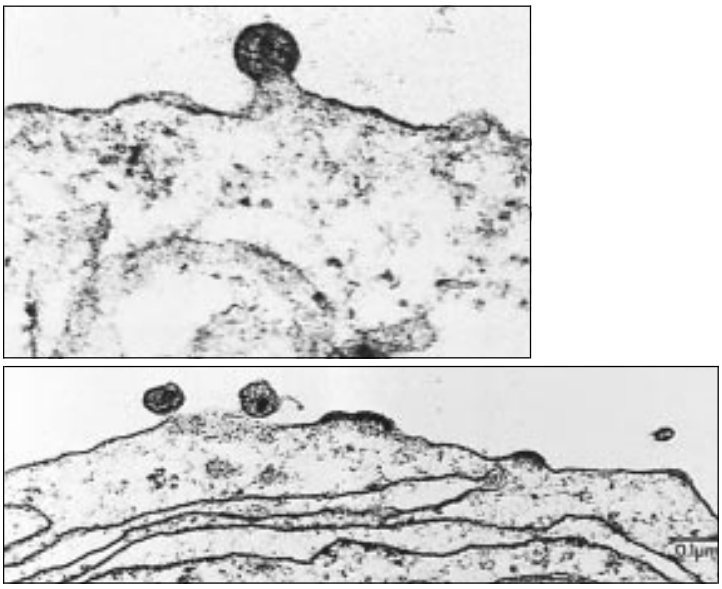

Fig 1 HIV virions entering cell (top) and budding from membrane (bottom) (reproduced with permission from Stein et al[1])

T cell subsets in the 1990s

Over the past 10 years many practitioners have become comfortable with the basic terminology to describe T cell subsets, which include CD4 (helper) and CD8 (cytotoxic) cells. Much more is now known about the range of functions of these cells. In 1986 Mosmann et al showed that CD4 T lymphocytes could be categorised according to the cytokines they made.[12] These subsets have distinct functional characteristics—T helper 1 (Th1) cells make interferon γ and are associated with cell mediated immunity, while T helper 2 (Th2) cells make interleukin 4 and help B cells to make antibody (table). Within the past three years comparable populations of CD8 T lymphocytes (called Tc1 and Tc2) have been identified in mice,[13 14] rats,[15] and humans,[16–18] with interferon γ secretion representing Tc1 T lymphocytes, interleukin 4 secretion representing Tc2 T lymphocytes, and an unrestricted cytokine profile representing Tc0 T lymphocytes. The functions of these different CD8 subsets have

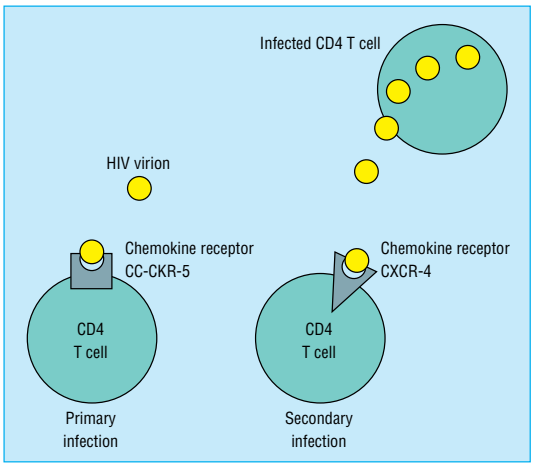

Fig 2 CC-chemokine receptors in primary and secondary infection with HIV

T cell subsets

Cell type	Cytokines produced	Major cell functions
T helper 1 (Th1)	Interferon γ	Activates macrophages and T cytotoxic 1 (Tc1) cells
		Has a major role in eradicating viruses
		Thought to be damaging cell type in autoimmune disease
		Inhibits T helper 2 (Th2) cells
T helper 2 (Th2)	Interleukin 4, interleukin 5	Promotes antibody production
		Promotes allergic disease
		Inhibits Th1 cells
T cytotoxic 1 (Tc1)	Interferon γ	Is efficient killer cell
		Inhibits Th2 cells
T cytotoxic 2 (Tc2)	Interleukin 4, interleukin 5	Is less efficient at killing
		Inhibits Th1 cells

yet to be fully defined, but some tantalising reports are beginning to appear in relation to human disease. Tc0 cells predominate in immune responses that are ineffective in clearing intracellular organisms, such as occurs in leprosy.[19] Tc2 lymphocytes secreting interleukin 4 have been found in people infected with HIV.[20] Since interleukin 4 inhibits Tc1 CD8 T lymphocytes,[15] which are likely to be a major part of the antiviral response,[21] this profile could have a severely deleterious effect on host immunity. In allergy, Tc2 CD8 T lymphocytes may provide help for IgE antibody responses[22] and promote airway inflammation through secretion of interleukin 5, which attracts inflammatory eosinophils.[23] As yet, there is no simple, one off blood test that can measure the ratio of Th1 to Th2 or Tc1 to Tc2.

Apoptosis: cell suicide

The immune system is the only organ to require massive cellular expansion within a period of hours in response to infection. This capacity brings with it some danger: activated cytotoxic cells, complete with toxic mediators and potent cytokines, must be controlled to avoid tissue damage by friendly fire. The removal of unwanted, activated cells is achieved by inducing them to commit suicide, a process termed apoptosis. Major advances in the understanding of the molecular events of apoptosis are beginning to offer new therapeutic options.

The main molecule concerned with the form of apoptosis used to regulate death of activated lymphocytes (activation induced cell death) is Fas (CD95), widely expressed on T and B lymphocytes.[24] Fas expression on these cells increases after encounter with antigen. Binding of Fas to its ligand leads to apoptosis of the cell expressing Fas through activation of an intracellular cascade of proteases. The expression of the Fas ligand is tightly regulated in the immune system, but it begins to be expressed on mature CD4 and CD8 T cells after activation. Thus, towards the end of an immune response the Fas ligand on activated T cells is able to bring about activation induced cell death in cells which have upregulated Fas (fig 3).

Understanding the mechanisms of activation induced cell death has had great bearing on some clinical problems.

Firstly, a rare early childhood syndrome characterised by lymphoproliferation with lymphadenopathy, autoimmune haemolysis, thrombocytopenia, and hypergammaglobulinaemia has recently been attributed to mutations in the Fas gene. Activation induced cell death in these patients is severely defective, allowing lymphoproliferation to go unchecked.[25 26]

Secondly, Fas expression has been noted on thyroid epithelial cells in patients with autoimmune thyroiditis.[27] Thyrocyte damage in this condition is thought to be due to apoptosis induced by the interaction between activated T lymphocytes bearing the Fas ligand and thyrocytes bearing Fas. This is a new explanation for the pathogenesis of destructive autoimmune disease and may allow new intervention strategies which interfere with the Fas system to be devised.

Finally, Fas may be important in transplantation. It has been known for many years that foreign grafts transplanted into certain sites (notably the eye and testis) are not rejected. The conventional explanation for this immune privilege has been a lack of lymphatics in these organs. However, the Fas ligand is permanently expressed in both the eye and the testis.[28] Thus the immune privilege in these sites may be attributed to the killing of any lymphocytes expressing Fas in response to the allograft by the cells with the Fas ligand. This fascinating possibility is already being developed therapeutically to protect allografts. In experimental animals, islet cell allografts wrapped in myocytes genetically engineered to express the Fas ligand are not rejected, whereas those wrapped in unmanipulated myocytes are.[29] Not all of the information about Fas and its ligand is good, however. Recent reports of cancer cells expressing the Fas ligand and inducing activation induced cell death in tumour specific cytotoxic T cells have lead to articles entitled "The tumour cell strikes back."[30]

Antigen recognition by T cells

T cell receptors for antigen do not bind soluble antigen like their antibody counterparts on B cells. Instead, an elaborate and complex process exists, whereby polypeptide antigens are internalised and processed by specialist antigen presenting cells, giving rise to short peptides. These in turn bind to molecules of the major histocompatibility complex and the combination is recognised by the T cell receptor. It is understandable, therefore, why in 1987 the immunology world was

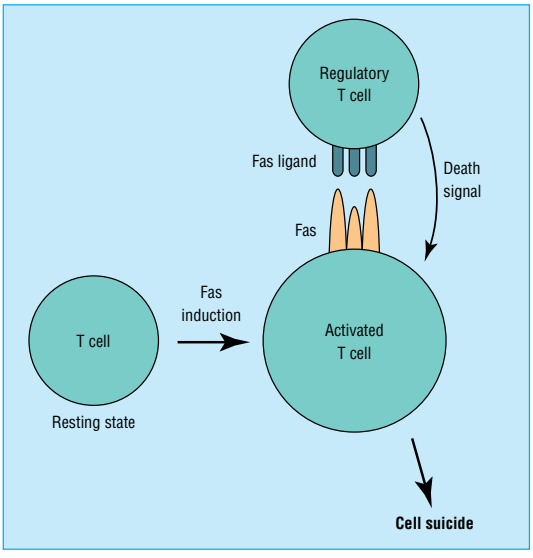

Fig 3 Activation induced cell death

captivated by the first *x* ray crystallographic study of the structure of a molecule of the major histocompatibility complex.[31] The crystal structure shows a long groove, perched on the backbone of the molecule, in which antigen derived peptides are embedded for recognition by the T cell receptor. Owing to difficulties in solubilising sufficient quantities of these receptors, the equivalent crystallographic view of a T cell receptor bound to peptide in complex with major histocompatibility complex has taken 10 years to be developed. The crystal structure shows that the T cell receptor sits diagonally over the groove in the molecule from the major histocompatibility complex.[32 33] As a consequence, large portions of the receptor interact with the walls of the molecule of the major histocompatibility complex itself. Only a small region of the receptor interacts with the peptide and then only with a small part of the peptide antigen. With this view, it is more understandable why foreign molecules of the major histocompatibility complex encountered in an allograft look so different and thus induce such potent T cell responses.

Functional studies on T cell recognition of antigen have also revealed some surprises. It was assumed for many years that T cells were activated in an all or none fashion, but by altering peptide antigens slightly it has become apparent that T cells have many grades of response, from activation to complete unresponsiveness (anergy).[34] Thus T cells behave just like a pharmacological receptor, with agonists, partial agonists, and antagonists. This has led to the possibility of using slightly altered peptides as ligands to switch off unwanted T cell responses—even damaging Th1 responses can be switched to protective Th2-like activity.[35] This and other developments in antigen recognition are important for diseases and treatments in which immune effectors have a role—for example, autoimmunity and immunisation.

It has now been shown unequivocally that a single T cell, reactive with a single self antigen, can also be stimulated by a peptide antigen from a virus.[36] In this study the T cells came from a patient with multiple sclerosis and were directed against myelin components from the central nervous system, but they could also be switched on by peptides from common viruses such as herpes simplex and Epstein-Barr. It is easy to see why the role of viruses in autoimmune disease is one of the hottest topics in immunology.

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1 Stein BS, Gowda SD, Lifson JD, Penhallow RC, Bensch KG, Engleman EG. pH-independent HIV entry into CD4 T cells via virus envelope fusion to the plasma membrane. *Cell* 1987;49:659-68.
2 Paxton WA, Martin SR, Tse D, O'Brien TR, Skurnick J, Van Devanter NL, et al. Relative resistance to HIV-1 infection of CD4 lymphocytes from persons who remain uninfected despite multiple high-risk sexual exposure. *Nature Med* 1996;2:412-7.
3 Dragic T, Litwin V, Allaway GP, Martin SR, Huang Y, Nagashima KA, et al. HIV-1 entry into $CD4^+$ cells is mediated by the chemokine receptor CC-CKR-5. *Nature* 1996;381:667-73.
4 Oberlin E, Amara A, Bachelerie F, Bessia C, Virelizier JL, Arenzana-Seisdedos F, et al. The CXC chemokine SDF-1 is the ligand for LESTR/fusin and prevents infection by T-cell-line-adapted HIV-1. *Nature* 1996;382:833-5.
5 Lehner T, Wang Y, Cranage M, Bergmeier LA, Mitchell E, Tao L, et al. Protective mucosal immunity elicited by targeted iliac lymph node immunization with a subunit SIV envelope and core vaccine in macaques. *Nature Med* 1996;2:767-75.
6 Lang DM, Polansky M. Patterns of asthma mortality in Philadelphia from 1969 to 1991. *N Engl J Med* 1994;331:1542-6.
7 Von Mutius E, Martinez FD, Fritzsch C, Nicolai T, Roell G, Thiemann HH. Prevalence of asthma and atopy in two areas of West and East Germany. *Am J Respir Crit Care Med* 1994;149:358-64.
8 Fischer P, Kozma P, Kalich R, Vandra E. Clinical findings in patients with lung diseases caused by non-tuberculous mycobacteria in former East Germany and Hungary. *Z Erkr Atmungsorgane* 1991;177:88-92.
9 Holt PG. Infections and the development of allergy. *Toxicol Lett* 1996;86:205-10.
10 Diaz-Sanchez D, Tsien A, Fleming J, Saxon D. Combined diesel exhaust particulate and ragweed allergen challenge markedly enhances human in vivo nasal ragweed-specific IgE and skews cytokine production to a T helper cell 2-type pattern. *J Immunol* 1997;158:2406-13.
11 Marsh DG, Neely JD, Breazeale DR, Ghosh B, Freidhoff LR, Ehrlich-Kautzky E, et al. Linkage analysis of IL4 and other chromosome 5q31.1 markers and total serum immunoglobulin E concentrations. *Science* 1994;264:1152-6.
12 Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL. Two types of murine helper T cell clones. I. Definition according to profiles of lymphokine activities and secreted proteins. *J Immunol* 1986;135:2348-57.
13 Croft M, Carter L, Swain SL, Dutton RW. Generation of polarized antigen-specific CD8 effector populations: reciprocal action of interleukin (IL)-4 and IL-12 in promoting type 2 versus type 1 cytokine profiles. *J Exp Med* 1994;180:1715-28.
14 Sad S, Marcotte R, Mosmann TR. Cytokine-induced differentiation of precursor mouse $CD8^+$ T cells into cytotoxic $CD8^+$ T cells secreting Th1 or Th2 cytokines. *Immunity* 1995;2:271-9.
15 Noble A, Macary PA, Kemeny DM. IFN-γ and IL-4 regulate the growth and differentiation of $CD8^+$ T cells into subpopulations with distinct cytokine profiles. *J Immunol* 1995;155:2928-37.
16 Wassenaar A, Reinhardus C, Abraham-Inpijn L, Kievits F. Type-1 and type-2 $CD8^+$ T-cell subsets isolated from chronic adult periodontitis tissue differ in surface phenotype and biological functions. *Immunology* 1996;87:113-8.
17 Birkhofer A, Rehbock J, Fricke H. T lymphocytes from the normal human peritoneum contain high frequencies of Th2-type $CD8^+$ T cells. *Eur J Immunol* 1996;26:957-60.
18 Zanussi S, Simonelli C, D'Andrea M, Caffau C, Clerici M, Tirelli U, et al. $CD8^+$ lymphocyte phenotype and cytokine production in long-term non-progressor and in progressor patients with HIV-1 infection. *Clin Exp Immunol* 1996;105:220-4.
19 Salgame P, Abrams JS, Clayberger C, Goldstein H, Convit J, Modlin RL, et al. Differing lymphokine profiles of functional subsets of human CD4 and CD8 T cell clones. *Science* 1991;254:279-82.
20 Maggi E, Giudizi MG, Biagiotti R, Annunziato F, Manetti R, Piccinni MP, et al. Th2-like CD8+ T cells showing B cell helper function and reduced cytolytic activity in human immunodeficiency virus type 1 infection. *J Exp Med* 1994;180:489-95.
21 Sad S, Mosmann TR. Interleukin (IL)-4, in the absence of antigen stimulation, induces an anergy-like state in differentiated $CD8^+$ Tc1 cells: loss of IL-2 synthesis and autonomous proliferation but retention of cytotoxicity and synthesis of other cytokines. *J Exp Med* 1995;182:1505-15.
22 Erard F, Wild MT, Garcia-Sanz JA, Le Gros G. Switch of CD8 T cells to noncytolytic $CD8^-CD4^-$ cells that make TH2 cytokines and help B cells. *Science* 1993;260:1802-5.
23 Coyle AJ, Erard F, Bertrand C, Walti S, Pircher H, Le Gros G. Virus-specific $CD8^+$ cells can switch to interleukin 5 production and induce airway eosinophilia. *J Exp Med* 1995;181:1229-33.
24 Van Parijs L, Abbas AK. Role of Fas-mediated cell death in the regulation of immune responses. *Curr Opin Immunol* 1996;8:355-61.
25 Fisher GH, Rosenberg FJ, Straus SE, Dale JK, Middelton LA, Lin AY, et al. Dominant interfering Fas gene mutations impair apoptosis in a human autoimmune lymphoproliferative syndrome. *Cell* 1995;81:935-46.
26 Rieux-Laucat F, Le Deist F, Hivroz C, Roberts IAG, Debatin KM, Fischer A, et al. Dominant interfering fas gene mutations impair apoptosis in a human autoimmune lymphoproliferative syndrome. *Science* 1995;268:1347-9.
27 Giordano C, Stassi G, De Maria R, Todaro M, Richiusa P, Papoff G, et al. Potential involvement of Fas and its ligand in the pathogenesis of Hashimoto's thyroiditis. *Science* 1997;275:960-4.
28 Suda T, Takahashi T, Golstein P, Nagata S. Molecular cloning and expression of the Fas ligand, a novel member of the tumor necrosis factor family. *Cell* 1993;75:1169-78.
29 Lau H, Yu M, Fontana A, Stoeckert C Jr. Prevention of islet allograft rejection with engineered myoblasts FasL in mice. *Science* 1996;273:109-12.
30 Strand S, Hofman WJ, Hug H, Muller M, Otto G, Strand D, et al. Lymphocyte appoptosis induced by CD95 (APO-1/Fas) ligand-expressing tumor cells—a mechanism of immune evasion. *Nature Med* 1996;2:1361-6.
31 Bjorkman PJ, Saper MA, Samraoui B, Bennett WS, Strominger JL, Wiley DC. Structure of the human class I histocompatibility antigen, HLA-A2. *Nature* 1987;329:506-12.
32 Sloan-Lancaster J, Evavold BD, Allen PM. Induction of T-cell anergy by altered T-cell receptor ligand on live antigen-presenting cells. *Nature* 1993;363:156-9.
33 Garboczi DN, Ghosh P, Utz U, Fan QR, Biddison WE, Wiley DC. Structure of the complex between human T cell receptor, viral peptide and HLA-A2. *Nature* 1996;384:134-41.
34 Nicholson LB, Greer JM, Sobel RA, Lees MA, Kuchroo VK. An altered peptide ligand mediates immune deviation and prevents experimental autoimmune encephalomyelitis. *Immunity* 1995;3:397-405.
35 Garcia KC, Degano M, Stanfield RL, Brunmark A, Jackson MR, Peterson PA, et al. An αβ T cell receptor structure at 2.5A and its orientation in the TCR-MHC complex. *Science* 1996;274:209-19.
36 Wucherpfennig KW, Strominger JL. Molecular mimicry in T cell-mediated autoimmunity: viral peptides activate human T cell clones specific for myelin basic protein. *Cell* 1995;80:695-705.

Lesson of the week

Central venous air embolism causing pulmonary oedema mimicking left ventricular failure

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Venous air embolism should be considered in the differential diagnosis of acute pulmonary oedema

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Central venous air embolism is recognised as a cause of acute cardiorespiratory collapse, and up to 50% mortality has been reported.[1] A rare and more insidious presentation is that of non-cardiogenic pulmonary oedema.

Case report

A 71 year old woman presented with irregular palpitations. Resting 12 lead electrocardiogram showed sinus rhythm at 95 beats per minute, first degree heart block (PR interval 360 ms), left axis deviation, and anteroseptal Q waves suggestive of a previous myocardial infarction. These results were similar to those seen in an electrocardiogram performed in 1991.

Asymptomatic episodes of complete atrioventricular block, frequent ventricular extrasystoles, and infrequent episodes of non-sustained ventricular tachycardia were found on Holter monitoring. She was admitted to hospital to await transfer for insertion of a pacemaker. An Arrowset 8.5 French gauge Swan-Ganz introducer (Arrow International, Reading, PA) was inserted into the right subclavian vein, and a 6 French gauge temporary pacing wire was passed through it. A chest radiograph taken after insertion was normal.

During the first 24 hours after insertion amiodarone hydrochloride 1325 mg was given through the side arm of the subclavian catheter followed by 200 mg orally daily thereafter for 5 days. Displacement of the pacing wire twice in one night occurred 6 days after insertion and necessitated repositioning. A stable paced rhythm of 80 beats per minute was achieved, but she developed clinical and radiographic evidence of pulmonary oedema within 4 hours of the pacing wire being repositioned. Repeated doses of diuretics were given intravenously over 6 hours but did not relieve the patient's symptoms. Because her condition continued to deteriorate, she was transferred to our cardiology unit. On arrival she was pale, dyspnoeic, anuric, sweating profusely, and centrally cyanosed despite treatment with high flow inspired oxygen given by mask. Her pulse was 80 and regular (ventricular paced rhythm) and her blood pressure was 110/80 mm Hg. There was no jugular venous distension visible above the sternal angle. Crackles were audible from the lung bases to the mid-zone.

A dual chamber permanent pacemaker was implanted as an emergency procedure to restore ventricular synchrony; it was assumed that this was contributing to the deterioration in her haemodynamic state. The temporary pacing wire was removed and the Swan-Ganz introducer was left in situ to maintain central venous access (fig 1). In spite of atrioventricular sequential pacing the patient became increasingly hypoxaemic (arterial oxygen saturation 89%) and hypotensive (blood pressure 70/35 mm Hg). Transthoracic echocardiography confirmed decreased anterior left ventricular wall motion consistent with previous myocardial infarction; overall left ventricular function was good and dimensions were normal. The right atrium and right ventricle seemed normal but frequent streams of echogenic air bubbles were seen, which confirmed the presence of a central venous air embolism (fig 2).

Inspection of the insertion site of the subclavian line showed that there was cyclical ballooning of the occlusive plastic dressing and incomplete apposition of the skin and subcutaneous tissue to the introducer.

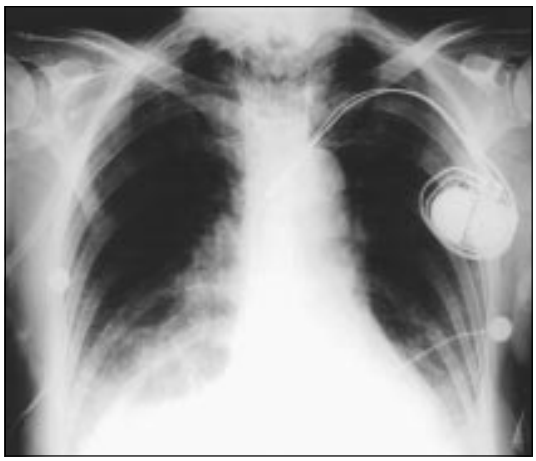

Fig 1 Chest radiograph taken before central venous catheter removed. The radiologist's report stated: dual lead cardiac pacemaker in situ; bilateral effusions and septal lines present at both bases are indicative of pulmonary oedema

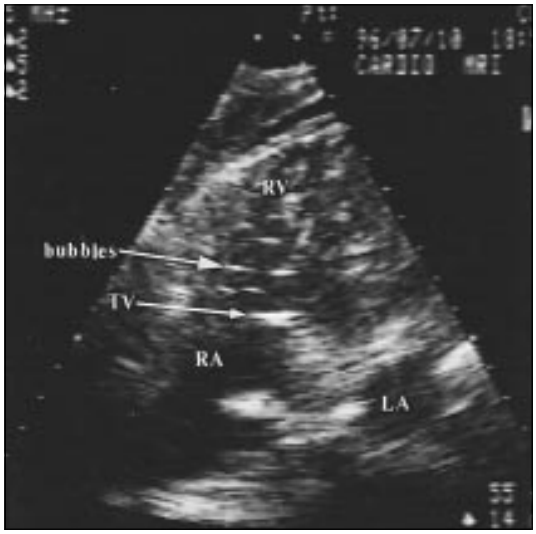

Fig 2 Apical view echocardiogram showing numerous air bubbles in the right heart. RA=right atrium, TV=tricuspid valve, RV=right ventricle, LA=left atrium

This suggested that air was being aspirated into the subclavian vein during respiration. The dressing formed a tent with the side arm of the introducer and seemed to have been acting as a one way valve. The introducer was removed immediately and an occlusive dressing applied. The patient was placed in a head down position; a triple lumen catheter was inserted into the right internal jugular vein to record central venous pressure and to allow drugs to be given. Central venous pressure was -2 cm H_2O with respect to the sternal angle when the patient was semirecumbent. Fluid resuscitation with colloid was started. Her condition improved rapidly over the next 2 hours. Thirty six hours later a chest radiograph showed complete resolution of the pulmonary oedema (fig 3). No further ventricular arrhythmias occurred, and treatment with amiodarone hydrochloride was stopped. The patient was discharged without taking diuretics.

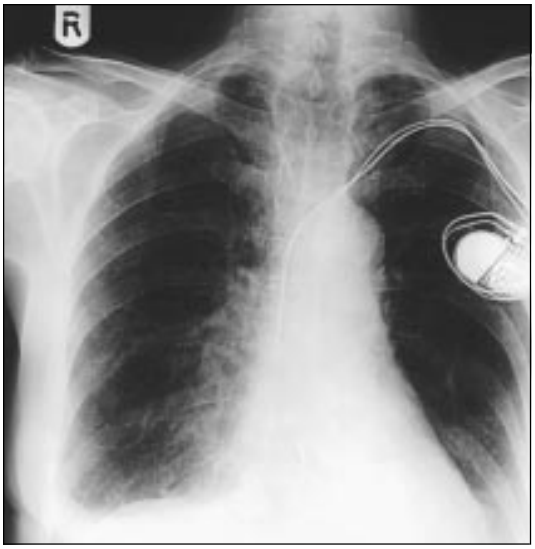

Fig 3 Chest radiograph taken 36 hours after removal of the introducer and reapplication of the occlusive dressing. The radiologist's report stated: the heart size is normal; there is a right pleural effusion and possibly a left pleural effusion; no evidence of pulmonary oedema

Discussion

In this case a central venous air embolism was suspected when air bubbles in the right heart were detected on echocardiography. There was no other clinical or echocardiographic explanation for a cardiac cause of this patient's pulmonary oedema. Her circulatory problems did not respond to atrioventricular sequential pacing but resolved rapidly after the central line was removed.

Acute pulmonary oedema occurring secondary to venous air embolism has rarely been reported[2]; its development seems to depend on a steady supply of small air bubbles into the pulmonary circulation which produces an acute inflammatory response in the pulmonary microvasculature. In a reported case of self injection of air, measurement of protein concentrations in fluid and plasma from pulmonary oedema confirmed that this oedema occurs secondary to hyperpermeability of the microvasculature.[3]

Most studies into the aetiology have, however, been performed in animals. Experimental induction of air embolism in sheep has shown that air bubbles in the pulmonary circulation attract large numbers of polymorphonuclear leucocytes.[4] Also in sheep, depletion of circulating leucocytes[5] or the addition of superoxide dismutase (which inactivates the toxic superoxide anion released by activated leucocytes)[6] reduces the pulmonary oedema. This led Staub and colleagues to conclude that the pulmonary oedema resulted from microvascular injury in the endothelium occurring secondary to leucocyte production and release of toxic oxygen metabolites.[4–6]

Electron spin resonance spectroscopy has been used in mice to show the role of free radicals derived from oxygen in acute lung injury by detecting increased production of free radicals at the time of increased permeability of the alveolar-capillary barrier.[7] In vivo the alveolar-capillary barrier consists of two juxtaposed surfaces: the capillary endothelium and the alveolar epithelium. The alveolar epithelium is the more impermeable of the two layers and maintains alveolar dryness through the removal of solute, including albumin, from the alveolus. By measuring increased clearance rates of aerosolised calcium trisodium pentetate (DTPA) labelled with technetium-99m, an indicator of the epithelial integrity of the alveolus, it is clear that hyperpermeability in acute lung injury is predominantly the result of damage to the epithelial layer of the alveolus.[8]

In sheep once the infusion of air is stopped a rapid return to baseline values occurs within 24 hours without additional treatment.[4] This is also the usual course in people, although in one reported case a patient required mechanical ventilation and in another a patient developed the adult respiratory distress syndrome.[2] Treatment should be supportive and include the use of oxygen, maintenance of a meticulous fluid balance, and the use of cardiorespiratory augmentation if required.

In this case we were alerted to the venous air embolism by the echocardiographic detection of intracardiac air bubbles. This finding resulted in prompt closure of the site of air entry, withdrawal of the diuretics which had contributed to the patient's hypovolaemia, careful fluid resuscitation, and prevention of a disastrous outcome. The use of echocardiography in the differential diagnosis of venous air embolism has been reported twice, and this case shows its effectiveness in diagnosing resultant pulmonary oedema.[9 10] Ultrasonography already has a defined role in monitoring haemodynamically silent air embolism during neurosurgical procedures; the ability to detect volumes of air as small as 0.3 ml confirms its usefulness as a highly sensitive tool.[10]

Venous air embolism should be considered in the differential diagnosis of acute pulmonary oedema; this is especially important in coronary care where central venous cannulation is common and pulmonary oedema may automatically be attributed to cardiac dysfunction, thus delaying appropriate treatment. Vigilance during the insertion of central venous catheters is essential if this potentially fatal complication is to be avoided: the size and depth of the skin incision should be minimised so that the cannula fits snugly, the insertion point should be covered by an impermeable dressing that adheres well, and the site should be inspected regularly.

1 Kashuk JL, Penn I. Air embolism after central venous catheterisation. *Surg Gynecol Obstet* 1984;159:249-52.
2 Kuhn M, Fitting JW, Levenberger P. Acute pulmonary edema caused by venous air embolism after removal of a subclavian catheter. *Chest* 1987;92:364-5.
3 Clark MC, Flick MR. Permeability pulmonary edema caused by venous air embolism. *Am Rev Respir Dis* 1984;129:633-5.
4 Okhuda K, Nakahara K, Binder A, Staub NC. Venous air emboli in sheep: reversible increase in lung microvascular permeability. *J Appl Physiol* 1981;51:887-94.
5 Flick MR, Perel A, Staub NC. Leukocytes are required for increased lung microvascular permeability after microembolisation in sheep. *Circ Res* 1981;48:344-51.
6 Flick MR, Hoeffel JM, Staub NC. Superoxide dismutase with heparin prevents increased lung vasculature permeability during air emboli in sheep. *J Appl Physiol* 1983;55:1284-91.
7 Murphy PG, Myers DS, Webster NR, Jones JG. Direct detection of free radical generation in an in vivo model of acute lung injury. *Free Radical Res Commun* 1991;15:167-76.
8 Murphy PG, Jones JG. Acute lung injury. *Br J Intensive Care* 1991;1:110-7.
9 Marcus RH, Weinert L, Neumann A, Borow KM, Lang RM. Venous air embolism: diagnosis by spontaneous right-sided contrast echocardiography. *Chest* 1991;99:784-5.
10 Kimura BJ, Chaux GE, Maisel AS. Delayed air embolism simulating pulmonary thromboembolism in the intensive care unit: role of echocardiography. *Crit Care Med* 1994;22:1884-6.

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Understanding controlled trials

What is Zelen's design?

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This is the fourth of an occasional series on the methods of randomised controlled trials

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When patients do not receive their preferred treatment in randomised trials there may be difficulties with patient recruitment and scientific problems with bias.[1] For example, bias may occur when patients are aware of a new treatment not available to them and comply poorly with the standard treatment.

Zelen's design can address these difficulties[2 3] by randomising patients *before* consent to participate has been sought. Two types of the design exist: double and single consent. In the double consent version patients are initially offered the treatment to which they were randomised; however, if they decline the randomised treatment, they can then be offered alternative therapies—including the experimental treatment. In the single consent version only patients offered the experimental treatment are told there is an alternative treatment (the control) available. Patients randomised to the control treatment are not allowed the experimental treatment (although they are given unhindered access to any usual treatment facilities). Analysis is undertaken with patients retaining their original assignment.

Zelen's design has been much discussed and for most therapeutic trials is probably unethical. Occasionally, however, it has been chosen on ethical grounds. For example, in a trial of extracorporeal membrane oxygenation for infants with pulmonary hypertension Zelen's design was used as it was considered preferable not to raise false hopes among half the parents that there was a novel treatment available for their child only to have it denied them through the randomisation.[4]

Zelen's design may be particularly useful for evaluating population based interventions such as screening, where it is important to estimate the effects on a whole population. However, if the presence of the trial is known to the non-screened group this may artificially induce changes in that group which may influence the results (a Hawthorne effect). For example, in a randomised trial of bone density screening[5] the non-screened group were not contacted at baseline as this might have artificially increased their use of hormone replacement therapy. Had the trialists not used Zelen's design the investigators could not have been sure of the full unbiased impact of screening on uptake of hormone replacement therapy.

If bias due to patients knowing they are in the "usual care" group is to be avoided patients usually need to be followed up for key events at a distance so as not to alert them to the study. For example, in a randomised trial of colorectal cancer screening cancer events for both groups of patients were ascertained through medical records and a cancer registry.[6] By using Zelen's design in screening trials it is possible to achieve more accurate estimates of population outcomes such as cancer reduction[6 7] compared with the conventional trial designs.

There are obvious ethical problems in using Zelen's design to randomise patients without their consent[8] (though treatment consent is always sought). For some interventions, however, such as screening, this may be the only practical design. For example, if all patients in the colorectal cancer screening trials had been screened but only a random half had been offered intervention, there would have been an ethical dilemma of not offering further investigation and treatment to control patients who appeared to be at high risk.

Zelen's design can have other disadvantages. If the trial requires intrusive data collection or monitoring then Zelen's design as control patients will be aware of the study. Given that intrusive data collection is not feasible, it may not be possible to use restrictive inclusive or exclusive patient recruitment criteria. Furthermore, if many patients refuse their original treatment, this will lead to a reduction in study power. Both these factors will lead to the need for a large sample size.[9]

1 Torgerson DJ, Sibbald B. What is a patient preference design? *BMJ* 1998;316:360.
2 Zelen M. A new design for randomized clinical trials. *N Engl J Med* 1979;300:1242-5.
3 Zelen M. Randomized consent designs for clinical trials: An update. *Stats in Med* 1990;9:645-56.
4 O'Rourke PP, Crone RK, Vacanti JP, Ware JH, Lillehli CW, Parad RB, et al. Extracorporeal membrane oxygenation and conventional medical therapy in neonates with persistent pulmonary hypertension of the newborn: A prospective randomized study. *Pediatrics* 1989;84:957-63.
5 Torgerson DJ, Thomas RE, Campbell MK, Reid DM. Randomised trial of osteoporosis screening: HRT uptake and quality of life results. *Arch Intern Med* 1997;157:2121-5.
6 Hardcastle JD, Chamberlain JO, Robinson MHE, Moss SM, Amar SS, Balfour TW, et al. Randomised controlled trial of faecal-occult-blood screening for colorectal cancer. *Lancet* 1996;348:1472-7.
7 Kronborg O, Fenger C, Olsen J, Jorgensen OD, Sondergaard O. Randomised study of screening for colorectal cancer by faecal occult blood test. *Lancet* 1996;348:1467-71.
8 Smith R. Informed consent: the intricacies. *BMJ* 1997;314:1059-60.
9 Altman DG, Whitehead J, Parmar MKB, Stenning SP, Fayers PM, Machin D. Randomised consent designs in cancer clinical trials. *Eur J Cancer* 1995;31A;1934-44.