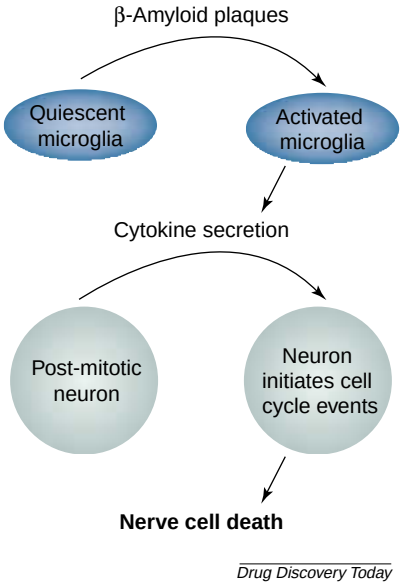

Figure 2. Proposed model for the progression of Alzheimer's disease[3].

– tumours originating from nerve cell division – are unknown in the adult, whereas you find tumours of almost every other tissue. Maybe the answer is that, in a way, you do find neuroblastoma in the adult, but we call it AD. The condition may be some kind of early cancer of the brain.'

New opportunities for AD therapy

This hypothesis enables new potential strategies for the treatment of AD. For example, one approach would be to block the signal that is responsible for initiating mitosis, either by reducing its concentration or by interfering with the neurons' response to it. Herrup says, 'We have ideas of what the signal might be, but there is no direct evidence for it yet. I am very partial myself to the inflammatory theory of AD, which says that a lot of the progression of AD, not the initiation, is due to chronic brain inflammation and activation of microglial cells. The cytokines that are released by these cells may actually be the source of the mitotic pressure that the nerve cells ultimately succumb to' (Fig. 2).

Another approach explored by Herrup and his team is to prevent the cells from re-entering the cell cycle, independent of what triggers them to do so. He concludes, 'Disregulation of the cell cycle may underlie more diseases than just cancer. Certainly, in terms of where we are looking in our attempt to block the effect of the mitotic pressure on these cells, we are going straight to the cancer literature where the work on the cell cycle has been extraordinary. That work is going to feed directly into what we are doing.'

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Serious infections stunt tumour growth

Kathryn Senior, Freelance writer

Severe infections could have anti-tumour properties that are independent of the specific immune system. Indeed, it has long been recognized that serious infections can slow or even halt tumour growth. Andrei Thomas-Tikhonenko (University of Pennsylvania, Philadelphia, PA, USA) and colleagues propose that infections might induce the upregulation of anti-angiogenic factors, which stunt cancer growth by cutting off the blood supply. Identifying the key factors responsible for this upregulation could, therefore, offer exciting new prospects for cancer therapy.

Historical observation

William B. Coley first observed that infection interferes with tumour growth in 1893. He described how streptococcal infection resulted in the regression of rare soft-tissue sarcomas in ten patients[1]. Subsequent studies showed that infection by several other pathogens also conferred host resistance to tumours and that the pathogens themselves could non-specifically activate macrophages to kill tumour cells *in vitro*. 'Despite the long-held belief that infection somehow stimulates the immune system, causing increased tumour surveillance, no cell-type or factor has ever been found to fully explain this phenomenon,' explains Thomas-Tikhonenko.

Is the immune system involved?

Thomas-Tikhonenko's group investigated the growth of B16F10 melanomas in mice infected by *Toxoplasma gondii*. The B16F10 cell line is only weakly immunogenic and causes rapidly growing tumours when injected subcutaneously in mice. As expected, mice infected by *T. gondii* on the day of tumour implantation developed much smaller tumours. By day 12 after implantation, the tumours

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in control mice had reached a mass of 0.5 g, but those in infected mice did not exceed 15 mg. 'These tumours were non-viable, with no real vasculature, and extensive areas of necrosis,' says Thomas-Tikhonenko. Mice deficient in various immune cells and factors were then used in similar experiments and the results were clear: exactly the same sort of inhibition of tumour growth was seen in mice infected by *T. gondii*, irrespective of the status of their immune system[2].

Infection inhibits angiogenesis

During the experiments by Thomas-Tikhonenko's group, a key finding was the lack of vasculature in the smaller tumours in infected mice and, therefore, the group decided to investigate the process of angiogenesis in control and infected animals. Histological examination showed that tumours from infected mice had no blood vessels, and that they showed no signs of endothelial cell infiltration. *In vivo* studies were then carried out using Matrigel pellets that consist of a semi-solid extracellular matrix-based compound, through which endothelial cells can infiltrate. The pellets were impregnated with basic fibroblast growth factor (bFGF) (an angiogenic stimulant), and implanted under the skin of mice, some of which were concurrently infected by *T. gondii*. After a week, the pellets were removed and the amount of blood in the pellets was measured. 'It was obvious that the pellets taken from infected mice were nearly white – those from control mice were bright red and oozed with blood,' recalls Thomas-Tikhonenko.

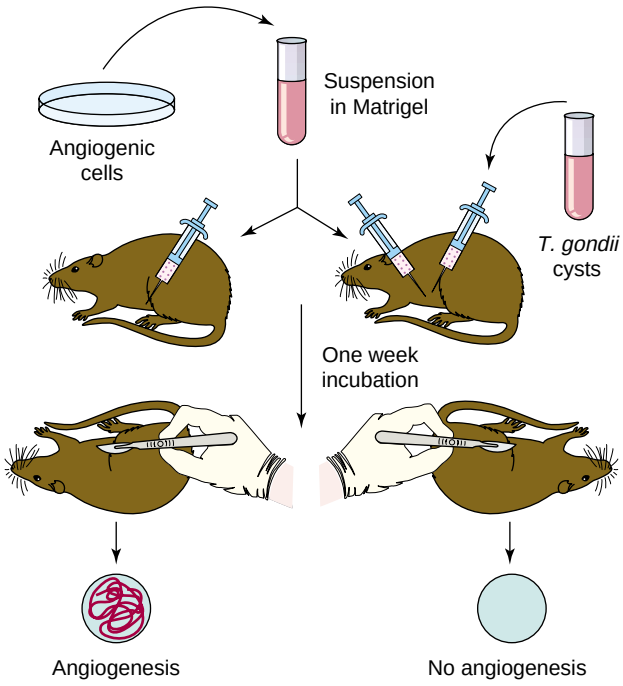

Figure 1. Lack of vasculature in small tumours of *Toxoplasma gondii* (*T. gondii*). Matrigel pellets consist of a semi-solid extracellular matrix-based compound, through which endothelial cells can infiltrate. The pellets were impregnated with basic fibroblast growth factor (bFGF) (an angiogenic stimulant), and implanted under the skin of mice, some of which were concurrently infected by *T. gondii*. After a week, the pellets were removed and the amount of blood in the pellets was measured. The pellets from the non-infected mice were highly vascularized, whereas those from the infected mice had little or no angiogenesis.

Searching out the elusive factor

Follow-up experiments have tried to identify anti-angiogenic factors that could be responsible. 'We started by looking at the factors that are known to inhibit blood vessel formation in tumours – interferon-γ and interleukin-12, for example – using a genetic approach,' says Thomas-Tikhonenko. So far, all knockout mice used have shown the same level of angiogenesis inhibition as controls, and the factor remains elusive. Currently, the group is planning to take large amounts of plasma from infected mice, fractionate it and then use an *in vitro* assay to refine the search. 'Normal endothelial cells seeded onto Petri dishes coated with Matrigel form a network of pseudocapillaries. By adding fractions from plasma we should be able to see if we get inhibition of blood-vessel formation,' he explains.

'The hypothesis that infection induces the expression or release of an angiogenesis inhibitor seems plausible since there have been demonstrations in animal models that one inhibitor alone – angiostatin or endostatin, for example – can inhibit tumour angiogenesis in animal models[3,4],' comments Michael Klagsbrun (Harvard Medical School, Boston, MA, USA). However, he cautions that: 'purification of such a factor from plasma, with its high concentration of protein, could be a formidable task, especially if more than one inhibitor were responsible for the effect.' Douglas Thompson (University of Aberdeen, Aberdeen, UK) also points out that even if a factor is identified, many hurdles would have to be overcome to exploit this finding to develop a future therapy. 'TNF-α, for example, appeared to be a fantastic inhibitor of angiogenesis, but was non-specific and caused death from shock when tested in an *in vivo* model[5],' he says.

Thompson also points out that interferons α and β were not specific enough for use in human patients and are toxic at high doses[6], although promising results were obtained in mice[7]. Nevertheless, he

adds that interferons have found niches in oncology for the treatment of certain leukaemias and lymphomas[8], and 'there may well be novel or existing inflammatory cytokines that could be used in combination therapies,' he says. Although Thomas-Tikhonenko admits that the search for an anti-angiogenic agent that will be useful in humans is still at the 'blue-skies' stage, he remains optimistic: 'A factor that can inhibit angiogenesis is something of a 'Holy Grail' – many are looking for it, perhaps we will be lucky.'

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Unravelling metabolic syndrome X

Sharon Dorrell, Freelance writer

A recent Canadian study reports an association between a rare autosomal dominant form of insulin resistance – Dunnigan-type familial partial lipodystrophy (FPLD) – and early coronary heart disease (CHD)[1]. According to Robert Hegele (Robarts Research Institute, London, Ontario, Canada), who carried out the study, this link is significant because the mutation of the *LMNA* gene that underlies FPLD and causes defects in the nuclear envelope also gives rise to a phenotype that resembles the insulin resistance syndrome (metabolic syndrome X; Box 1). A better understanding of FPLD could shed light on the mechanisms underlying insulin resistance in the general population, and perhaps eventually lead to improved treatments.

LMNA mutations

LMNA encodes for lamin A and C, which are components of the nuclear lamina, the mesh-like structure on the inner surface of the nuclear envelope. Two rare missense mutations in *LMNA* (R482Q and R482W) were discovered in Canadian FPLD families. Hegele's study found a higher prevalence of CHD among 23 heterozygous *LMNA* missense mutation carriers, all of whom were insulin resistant. Eight patients had CHD compared with only one of 17 homozygous family control subjects. Moreover, six of the patients with CHD had experienced heart attacks, coronary artery bypass grafts, or other CHD endpoints by the age of 55. The women in the group appeared to be particularly susceptible to these early CHD events.

'The *LMNA* mutations probably do not directly cause atherosclerosis,' says Hegele. 'Instead, the mutations appear to affect fat cells only, but in a very specific way that gives rise to the characteristic presentation of FPLD.' FPLD first becomes evident at the onset of puberty when those affected begin to lose fat from their extremities and the gluteal region until no fat is stored at these sites. Their central fat stores in the abdomen, face, neck, and shoulders are, however, unaffected and become the only place fat can be stored. Consequently, says Hegele, 'when people with this condition gain weight, the only place for the fat to accumulate is centrally and, as adults, these people exhibit an extreme form of central obesity.'

Hegele adds that he cannot yet explain why women with FPLD appear to be more susceptible to CHD events than men. It has been known for a while that diabetes eliminates the protection from atherosclerosis enjoyed by premenopausal

Box 1. Insulin resistance and metabolic syndrome X

In insulin-resistant individuals, insulin fails to stimulate glucose uptake and hyperinsulinaemia ensues to maintain glucose balance. Hyperinsulinaemia is associated with a cluster of metabolic complications that increase the risk of coronary heart disease, including glucose intolerance, dyslipidaemia and hypertension. Together, these complications are known as the insulin resistance syndrome or metabolic syndrome X, a syndrome that often occurs in people with central obesity.

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