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CAMPATH: from concept to clinic

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Lymphocyte depletion has a long history in the area of therapeutic immunosuppression. CAMPATH-1H (alemtuzumab) was generated in an attempt to replace anti-lymphocyte globulins in the transplant arena. Its efficacy in killing lymphocytes has established it as a licensed drug for the management of chronic lymphocyte leukaemia. Short-term therapy with alemtuzumab has demonstrated long-term benefit in a number of autoimmune conditions. This drug has the potential to facilitate recruitment of tolerance processes so enabling drug minimization in transplantation, autoimmune and hypersensitivity diseases.

Keywords: monoclonal antibodies; lymphocyte depletion; tolerance; drug minimization

1. INTRODUCTION

The discovery that lymphocytes were the principal mediators of acquired immunity (Gowans *et al.* 1962) and responsible for the rejection of allografts (McGregor & Gowans 1964) led to the search for anti-lymphocyte sera as agents to ensure allograft survival (Lance & Medawar 1968). Early studies in rodents demonstrated that lymphocyte depletion can indeed delay allograft survival, and even enable transplantation tolerance in selected models (Lance & Medawar 1969).

Ensuing studies in dogs (Kolb *et al.* 1973), non-human primates (Lance & Medawar 1970) and eventually man led to the emergence of anti-lymphocyte globulins as valuable agents for induction therapy.

The demonstration that lymphocyte-mediated graft versus host disease (GVHD) was a major impediment to allogeneic marrow transplantation also led to hopes that anti-lymphocyte antisera might be used to purge marrow if stem cells could be reliably spared (Weiden *et al.* 1979; Haas *et al.* 1980).

The seminal discovery of methodology for generating monoclonal antibodies (Mabs) in 1975 (Kohler & Milstein 1975) led to the development of reagents that could demarcate lymphocyte populations. Not only that, but it was clear that certain anti-T cell Mabs, of appropriate isotype, could be exploited as agents to kill lymphocytes *in vivo* (Cobbold *et al.* 1984). This new technology enabled a clear resolution of the issue as to which of the two major subsets of T cells (the CD4 helper cell, or the CD8 cytotoxic T cells) could mediate graft rejection and GVHD. The extent to which any subset might be involved seemed to be determined by the particular genetic combinations of donor and host (Korngold & Sprent 1985; Cobbold *et al.* 1986).

The observation that both CD4 and CD8 T cells were sufficient to mediate graft rejection and GVHD in murine models led to the acceptance that control of both these subpopulations would be required if anti-lymphocyte Mabs were to become reliable immunosuppressive agents.

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2. THE SEARCH FOR AN ANTI-LYMPHOCYTE ANTIBODY FOR THE *IN VITRO* PREVENTION OF GVHD

We reasoned that the pressing need to control GVHD would be met if we could identify an anti-lymphocyte antibody (preferably anti-T cell) capable of fixing human complement. This would permit controlled T cell lysis with donor complement *in vitro* prior to marrow infusion. In discussions with colleagues in those early days, it was not obvious that a single rat monoclonal antibody would have the ability to activate human complement. In contrast, polyclonal anti-lymphocyte antisera, which contained many antibody specificities, were expected to coat lymphocytes with sufficient antibody so as to activate C1, the first component of complement.

The scepticism was merited. A number of fusions following shotgun immunization of rats with human lymphocytes led to the discovery of just one set of antibodies competent to selectively kill human lymphocytes with human complement (Hale *et al.* 1983). We came to refer to this series as the Cambridge Pathology 1 or CAMPATH-1 antibodies. CAMPATH-1 antibodies were found to react with a GPI-anchored dodecapeptide (Xia *et al.* 1991), now known as CD52, which turns out to be one of the most abundant proteins on the lymphocyte surface. Anti-CD7 and anti-CD2 Mabs also proved lytic, but only on activated T cells. Early studies using the best available clonogenic assays for human blood stem cells suggested that our antibodies spared such 'stem' cells (Hale *et al.* 1983). Stem-cell sparing by was also demonstrated by transplanting *in vitro* purged autografts in non-human primates (Gerritsen *et al.* 1988). However, none of these studies were proof that CAMPATH-1 antibodies would avoid damage to the 'real' blood stem cells.

IgM antibodies were more efficient (as predicted) in lysing lymphocytes with human complement than were the IgG forms. One of these rat IgM reagents (CAMPATH-1M) was chosen for the initial clinical

pilot study. The first patient to have an allogeneic marrow transplant purged with CAMPATH-1M was suffering from aplastic anaemia. The purging process was quite simple using a small quantity of donor serum as a complement source. Gratifyingly, the patient responded with restoration of her blood system. However, we were stunned when analysis of the regenerating blood system showed that the reconstituting blood system was derived from her own stem cells, and not those of the donor. This good outcome for the patient left us with real concerns as to whether stem cells were indeed spared by CAMPATH-1M. After all why had we detected no donor reconstitution? Fortunately, our agony was short. The issue was soon resolved in a study conducted with Shimon Slavin at the Hadassah Hospital in Jerusalem. CAMPATH-1M-purging in a small cohort of patients receiving allogeneic transplants was followed by donor engraftment associated with only minor GVHD, despite the absence of added maintenance immunosuppression (Waldmann *et al.* 1984).

The sense of relief proved short-lived! It had become apparent that some patients who had initially engrafted were losing the graft (Waldmann *et al.* 1984). If this was not due to stem cell loss, then it must have been immunological. We speculated that the conventional conditioning that recipients were receiving could not have ablated all host immune function, leaving some capacity to reject. We reasoned that conventional immunosuppression and donor-mediated GVHD would, in conventional practice, have overridden this residual host propensity for rejection.

3. BACK TO THE DRAWING BOARD! MODELLING MARROW REJECTION IN THE MOUSE

This speculation led to the inevitable conclusion that we might need to control that residue of host alloreactivity. Could this be done by further ablating host lymphocytes with a lytic antibody? We established a murine model where the host was conditioned with a sub-lethal dose of irradiation. This allowed us to examine whether a small number of residual T cells could reject donor marrow, and whether additional host T cell ablation could prevent marrow rejection. We had generated a series of rat antibodies of the IgG2b isotype directed to murine T cells and subsets thereof. Ablation of both CD4 and CD8 T cells was, in these circumstances, able to permit marrow engraftment across an MHC barrier (Cobbold *et al.* 1986). We reasoned that monoclonal antibody ablation of host T cells would circumvent the rejection problem in the clinic. We needed to find a monoclonal anti-lymphocyte antibody capable of effective depletion *in vivo* in humans.

4. AN ETHICAL ENTRY POINT FOR EVALUATING THE LYTIC POTENTIAL OF CD52 ANTIBODIES IN THE CLINIC

We wished to know if any of the CD52 antibodies known to fix complement *in vitro* could lyse human lymphocytes *in vivo*. We teamed up with our local haematologists who themselves were seeking treatments for patients with refractory lymphocytic leukaemias and lymphomas. This was a fortunate avenue, as it led to the application of CD52 antibodies to lymphoid malignancies, the therapeutic area from which alemtuzumab emerged as a licensed drug. A patient with a prolymphocytic leukaemia proved enormously valuable in identifying a variant of a CD52 antibody able to substantively kill lymphocytes *in vivo*. We had observed that CAMPATH-1M and a complement fixing rat IgG2a anti-CD52 antibody were unable to debulk leukaemic cells, despite their ability to fix complement *in vitro* (Dyer *et al.* 1989). Clearly, complement fixation was insufficient for the task. We knew from the studies of rat Mabs as lytic agents in mice, and from *in vitro* studies of cell-mediated antibody dependent cytotoxicity (ADCC), that the rat IgG2b isotype was by far the most effective isotype in harnessing the cell-mediated lytic mechanisms (Clark *et al.* 1985). Our library of antibodies from shot-gun fusions contained no rat IgG2b forms of a CD52 antibody.

5. THE GENERATION OF CAMPATH-1G, A RAT IgG2b CD52 ANTIBODY

We adopted a number of different strategies to acquire an IgG2b CAMPATH-1 Mab. We set about cloning the rat immunoglobulin genes, if need be, to produce such an IgG2b form by antibody engineering. From this work, we learnt that the IgG2b locus was downstream of the IgG2a (Bruggemann *et al.* 1986). Natural class-switch variants might be predicted to occur, although none had yet been described for rat hybridomas. Using sensitive detection assays for single cell mutants, we were eventually able to derive a ridiculously rare class-switch variant (Hale *et al.* 1987). This antibody, which became known as CAMPATH-1G, was manufactured to clinical grade, and then infused into our patient who had been refractory to the IgM and IgG2a variants. Remarkably, leukaemic cells underwent substantial clearance from the blood (Dyer *et al.* 1989). We had a monoclonal antibody with potent lympholytic ability, one that might, in time, serve as the alternative to polyclonal anti-lymphocyte globulins!

6. THE EMERGENCE OF THE 'CAMPATH USERS GROUP' AND ITS ROLE IN DEMONSTRATING THAT CAMPATH-1G COULD PREVENT MARROW REJECTION

The incidence of marrow rejection in HLA-matched transplants had been such that a study to demonstrate its control would require a trial in a sizeable number of patients. In addition we could not be certain about which might turn out to be the most effective and user-friendly protocol to control both GVHD and marrow rejection simultaneously. A number of centres in the UK, Europe, Israel, Australia and South Africa came together to form an informal 'Users group'. This group had access to sufficient patients to be able to evaluate a number of different lymphocyte purging protocols with the CAMPATH-1M and CAMPATH-1G Mabs. These included protocols using CAMPATH-1M *in vitro* combined with CAMPATH-1G *in vivo*, or CAMPATH-1G 'in the bag', to CAMPATH-1G administered *in vivo* only (Willemze *et al.* 1992;

Jacobs *et al.* 1994; Hale & Waldmann 1996; Hamblin *et al.* 1996). These collaborative studies demonstrated that lymphocyte ablation was sufficient to reduce the incidence of GVHD and marrow rejection (Hale *et al.* 2001). They set the scene for research to minimize the intensity of host conditioning in marrow transplantation through use of non-myeloablative protocols sometimes referred to as mini-transplants (Hale *et al.* 2002; Perez-Simon *et al.* 2002). Clinical grade antibodies were produced initially in Cambridge, and then in Oxford at a purpose built facility known as the Therapeutic Antibody Centre. This centre, one of the only academic facilities of its kind, has provided CAMPATH-1 and other Mabs for clinical studies, including CD3, CD4, CD18, CD45 and many other therapeutic candidates.

7. THE ISSUE OF IMMUNOGENICITY, AND THE HUMANIZATION OF CAMPATH-1H

Although CAMPATH-1G was clearly active in the human, rodent studies suggested that cell-binding antibodies, especially those that were lytic, were likely to be immunogenic (Bruggemann *et al.* 1989). Those data were confirmed years later with rat Mabs given to man (Rebello *et al.* 1999). On the basis of our rodent studies alone we took the decision to create a human form of the CAMPATH-1G antibody. Engineering chimeric antibodies whose variable regions were rat derived and constant regions of human origin was one option. However, we had learned of Greg Winter's strategy for humanizing the variable regions, and opted to go that route in collaboration with his laboratory. If we were to humanize we needed to make a choice of IgG subclass to ensure good effector function. From our *in vitro* studies on complement lysis and ADCC, human IgG1 seemed the best choice of an Fc framework (Bruggemann *et al.* 1987; Riechmann *et al.* 1988). The engineered human IgG1 Mab (CAMPATH-1H) was then manufactured to clinical grade for therapeutic use.

8. CAMPATH-1H RETAINED LYMPHOLYTIC ACTIVITY FOR NEOPLASTIC AND NORMAL LYMPHOCYTES

Having manufactured a small quantity of CAMPATH-1H, we had an early opportunity to evaluate its lytic potential in a patient with non-Hodgkin's lymphoma.

We observed that a relatively small amount of antibody achieved a massive reduction in tumour load (Hale *et al.* 1988), and this exciting outcome was enough to set CAMPATH-1H on the road to becoming a drug for targeting lymphocyte neoplasms. Soon after, the late Martin Lockwood approached us about a young patient who was severely ill with a refractory vasculitic syndrome. This patient was given a relatively small amount of antibody, and again, we were gratified that the patient was able to experienced a long-lasting remission of her illness (Mathieson *et al.* 1990). This success in a single patient set the scene for the academic effort to establish CAMPATH-1H as a useful agent for induction therapy in autoimmune disease and in transplantation.

With Martin Lockwood we examined the utility of CAMPATH-1H in the treatment of the vasculitides (Lockwood *et al.* 1996). With Alastair Compston and Alasdair Coles, we have studied the potential of the drug as a treatment for late stage progressive multiple sclerosis (Coles *et al.* 1999), and more recently, for relapsing-remitting disease. With Peter Friend and Roy Calne we determined that CAMPATH-1H was a potent agent to reverse rejection episodes in organ transplantation (unpublished). In the course of these studies we showed that CAMPATH-1H was indeed far less immunogenic than CAMPATH-1G so retrospectively justifying the humanization of the drug (Rebello *et al.* 1999).

The CAMPATH users group also took the antibody on board, and introduced it into a range of protocols to prevent GVHD and marrow rejection (e.g. Hale *et al.* 2001; Kottaridis *et al.* 2001).

9. THE COMMERCIAL DEVELOPMENT OF CAMPATH-1H

From the outset, we could not possibly have predicted the haphazard and tortuous path which CAMPATH-1H had to take to become a licensed drug. British Technology group was assigned the rights to our CD52 antibodies by Cambridge University. These they licensed to Wellcome Biotech, who in turn were subsumed into Wellcome PLC. Wellcome PLC then merged with Glaxo to become Glaxo-Wellcome. Glaxo-Wellcome carried out trials which confirmed the value of CAMPATH-1H in the treatment of chronic B-cell leukaemia (BCLL), but could not see the drug competing in the lymphoma market, nor as an immunosuppressant in the rheumatoid arthritis market especially given the emerging success of anti-TNF therapies. They stopped their development of CAMPATH-1H in 1994.

At that time H.W. was moving to Oxford, and was seeking ways to raise funds to establish the therapeutic antibody facility in Oxford. A newly formed USA biotechnology company Leukosite, Inc. became interested in CAMPATH-1H, and consequently joined up with the Medical Research Council to fund the construction of the Oxford Therapeutic Antibody Centre. This enabled G.H. to move from Cambridge to manage that facility. Leukosite partnered with another biotechnology company ILEX, to develop CAMPATH-1H. A marketing partner (Schering AG) made up a triumvirate aiming to develop the drug. In July 2001 CAMPATH-1H, newly named alemtuzumab, was approved by the FDA for the treatment of fludarabine-resistant BCLL (Keating *et al.* 2002).

By that time Leukosite had been acquired by Millennium Pharmaceuticals. Millennium sold their rights to CAMPATH-1H to ILEX. In 2004 ILEX was acquired by Genzyme Corporation. Through the acquisition of Sangstat, Genzyme had also acquired the rights to sell Thymoglobulin (ATG; anti-thymocyte globulin), a polyclonal immunoglobulin preparation widely used as an immunosuppressant in transplantation.

Given that we were hoping that CAMPATH-1H would replace ATG as an induction agent, this situation was a real blow to our aspirations. To resolve the potential conflict of a company marketing two

competing products for identical indications, Genzyme transferred to Berlex/Schering AG the exclusive responsibility for development and commercialization of CAMPATH in solid organ transplantation. At the time of writing this chapter the extent to which Schering will take on this function remains unknown. Trials initiated by ILEX in multiple sclerosis should soon be reported, and decisions on possible commercial development for this disease will need to be taken.

10. CAMPATH-1H AS INDUCTION THERAPY IN ORGAN TRANSPLANTATION

The potency of CAMPATH-1H as a lympholytic agent had left some concerns about its use as induction therapy in transplantation. However, studies with CD3 immunotoxins in primates (Knechtle *et al.* 1997) suggested that T cell depletion might have a useful role in preparing the way for innovative tolerance-inducing strategies. A study examining the combined use of CAMPATH-1H induction and cyclosporin monotherapy did indeed live up to these expectations (Calne *et al.* 1999; Watson *et al.* 2005).

These promising results with lymphocyte depletion have now attracted much interest (Ciancio *et al.* 2004; Knechtle *et al.* 2004; Gruessner *et al.* 2005). Kirk and his colleagues have established unequivocally that lymphocyte depletion with short-term administration of CAMPATH-1H is insufficient to prevent rejection of renal allografts, albeit with little evidence of lymphocyte infiltrates (Kirk *et al.* 2003). The recent awareness that lymphocyte numbers are homeostatically controlled, and may rebound aggressively through 'homeostatic' expansion has tempered enthusiasm for lymphocyte depletion without some means to control the 'rebound'. It may be that calcineurin inhibitors provide that necessary dimension. Needless to say there should be a systematic analysis of what drugs can be used to enable the reconstituting immune system to 'reboot' correctly. The longer-term disadvantages of prolonged administration of calcineurin inhibitors may be offset by protocols that aim to wean patients off these drugs (Gruessner *et al.* 2005). Preliminary data suggest that weaning may elicit rejection episodes in some patients (Shapiro *et al.* 2005). For weaning to succeed we need to be able to identify patients who have achieved the status of low risk of rejection, or at least have in place early warning systems of rejection, so that impending rejection episodes can be aborted before they ignite.

Alemtuzumab induction has opened up opportunities for drug minimization protocols with steroid sparing as a likely immediate candidate (Axelrod *et al.* 2005; Gruessner *et al.* 2005). It may also create opportunities in 'tough' arenas such as gut and multi-organ transplantation where alloreactivity has hitherto been difficult to control (Tzakis *et al.* 2004; Tzakis *et al.* 2004; Kato *et al.* 2005). The enthusiasm and creativity of the transplant community will inevitably establish the correct context in which this drug can be used, and the manner of its use. In particular, the current cost of CAMPATH-1H, which has been priced for the treatment of BCLL, is very competitive with ATG or ALG.

11. CONCLUDING REMARKS

There can now be little doubt that lymphocyte-depleting strategies are attracting the attention of the transplant community. The potential of CAMPATH-1H in this arena is really in its early stages, and future development requires that we eliminate many of the prejudices that currently constrain its use. Lymphocyte depletion combined with an intact system of innate immunity may not pose the same risks as drugs that damage both adaptive and innate immunity. T cell and B-cell depletion may carry a lower risk of lymphoproliferative disease than T cell depletion alone. The maintenance-drug requirements to control a rebooting immune system may be far less than that needed to control an unspoilt immune system. Most important, the standardized and predictable activity of CAMPATH-1H must eventually weigh strongly against the potential variability of polyclonal animal antisera. Surely the days are numbered for such crude reagents.

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