The fruits of cooperation

Ann Hill and Helen Chapel

THREE weeks ago, *Nature* published an account of the identification of the gene responsible for the human primary immunodeficiency disease, X-linked agammaglobulinaemia (XLA)[1]. By careful genetic mapping of a disease locus, Vetrie and coworkers discovered a previously unknown protein-tyrosine kinase gene involved in B-cell development.

Now, five papers have popped up almost simultaneously describing how the opposite pathway has led to description of the cause of another X-linked immunodeficiency disease; two of the reports appear in this issue (pages 539[2] and 541[3]), the others in *Science*[4], *Cell*[5] and *Proceedings of the National Academy of Sciences*[6]. The disorder is X-linked immunodeficiency with hyper-IgM (HIGM1), a disease characterized by failure of T–B cell cooperation. It was knowledge of the essential part in the T–B cell cooperation played by the newly discovered CD40 ligand that resulted in identification of this gene as the culprit.

The interdependence of T and B cells has long been recognized, giving rise to the designation of a subclass of T cells as 'helpers' because of their obligatory role in enabling B cells to produce antibodies of different isotypes and to establish immunological memory. Both soluble factors (cytokines) and cell–cell contact are required for effective help. Cell–cell contact achieves the critical cross-linking of the B-cell activation marker, CD40. This enables B cells to proliferate and, in the presence of appropriate cytokines, to 'switch' the isotype of immunoglobulin they produce from IgM to IgG, IgA or IgE. CD40, apparently a member of a growing family of receptors related to the tumour necrosis factor (TNF) receptors[7], was until recently a receptor without a known ligand. Then, within the past six months, three groups reported the cloning of its murine and human ligands[8–11]. The CD40 ligand, it turned out, is a homologue of TNF-α and is expressed on the surface membrane of activated $CD4^+$ (helper T cells) as well as on some $CD8^+$ T cells.

A crucial lead in the story told in the new papers was the mapping of the gene for the CD40 ligand to the long arm of the X chromosome (Xq26.3–27.1)[4,9]. Also known to map in this region (Xq24–27) was the unidentified gene responsible for HIGM1[12,13]. This is a rare primary immunodeficiency syndrome characterized by the inability of B cells to undergo isotype switching: B cells produce normal amounts of IgM appropriately in response to antigenic challenge, but fail to switch to produce IgG, IgA and IgE and to establish memory. T-cell dysfunction was suspected in the disease because, unlike other antibody-deficient patients, some HIGM1 patients are surprisingly prone to certain infections commonly associated with T-cell deficiency, such as *Pneumocystis carinii* pneumonia and cryptosporidial diarrhoea. T cells were further implicated by an elegant study which demonstrated that HIGM1 B cells could be made to produce normal amounts of IgG, IgA and IgE if co-cultured with a malignant human T-cell line[14].

With this background, the five groups[2–6] set out to investigate the role of the CD40 ligand in HIGM1. They looked at a total of 18 unrelated individuals with HIGM1. Activated T cells from 17 of these individuals failed to bind an engineered soluble form of CD40; in the eighteenth, only weak binding was seen[4]. The CD40 ligand genes from 13 patients were then sequenced and in 12 a different point mutation or deletion was discovered. DiSanto *et al.*[3] show that, in three of their cases, the defective gene is carried by the asymptomatic mother. In a fourth case the defect appeared to be a new mutation. Four of the groups provide experimental evidence that the functional defect in B cells seen in HIGM1 is indeed due to failure to cross-link CD40. Cross-linking of CD40 restores the ability of HIGM1 B cells to proliferate and to undergo isotype switching when co-cultured with appropriate cytokines[2,4–6].

As icing on the cake, three of the groups describe experiments demonstrating that the defective CD40 ligand gene is indeed responsible for the T cells' inability to bind CD40. DiSanto *et al.*[3] take advantage of a polymorphic microsatellite repeat in the 3′ untranslated region of the CD40 ligand that they have identified. The mother of one patient carried two allelic forms of this marker. Because X-chromosome inactivation is random in the HIGM1 carrier, only half of her activated T cells express functional CD40 ligand: all of the T cells that failed to bind soluble CD40 displayed the microsatellite marker associated with the defective CD40 ligand gene. Allen *et al.*[4] have transfected cell lines with wild-type CD40 ligand genes or with identified HIGM1 mutant alleles. Whereas the wild-type transfectants bound CD40 and induced isotype switching in normal B cells, the lines transfected with the mutants failed to do so. Aruffo *et al.*[5] report similar results with soluble forms of the mutant CD40 ligand.

So knowledge of the pivotal importance of the CD40–CD40 ligand interaction in B-cell differentiation and isotype switching has led rapidly to identification of the CD40 ligand gene as responsible for HIGM1. A closer study of the phenotype of the disease now prompts further questions about the function *in vivo* of this ligand–receptor pair. There are two characteristic features of HIGM1[13] which to our minds are not easily explained by the currently identified role for the CD40 ligand in T–B cell cooperation. One is the mechanism by which inadequate T-cell function fails to control intracellular parasites such as *Pneumocystis carinii*; further study of HIGM1 T cells may help to shed light on this. It may be relevant that CD40 is expressed on epithelium, interdigitating cells and basophils as well as on B cells; to date, the CD40 ligand has been identified only on T cells. The other, even more puzzling, feature is the intermittent drop in granulocyte numbers which affects about half of the sufferers from this disease. Is the CD40 ligand involved in this in some way or is this really an autoantibody problem?

For HIGM1 patients and clinicians, carrier detection will be an immediate benefit. Specific therapies, with genes or recombinant proteins, are now possible, although most patients remain well with adequate antibody replacement. The benefits of human gene therapy and the outcome of animal trials with the recombinant proteins are eagerly awaited. For scientists, the heterogeneity of primary immunoglobulin deficiencies[5], in both phenotype and inheritance patterns, provides natural opportunities for identifying genes involved in the basic biology of antibody production. □

Ann Hill is in the Molecular Immunology Group, Institute of Molecular Medicine, and Helen Chapel is in the Nuffield Department of Medicine and the Department of Immunology, John Radcliffe Hospital, Headington, Oxford OX3 9DU, UK.

1. Vetrie, D. *et al. Nature* **361**, 226–233 (1993).
2. Korthäuer, U. *et al. Nature* **361**, 539–541 (1993).
3. DiSanto, J. P., Bonnefoy, J. Y., Gauchat, J. F., Fischer, A. & de Saint Basile, G. *Nature* **361**, 541–543 (1993).
4. Allen, R. C. *et al. Science* **259**, 990–993 (1993).
5. Aruffo, A. *et al. Cell* **72**, 291–300 (1993).
6. Fuleihan, R. *et al. Proc. natn. Acad. Sci. U.S.A.* (in the press).
7. Stamenkovic, I., Clark, E. A. & Seed, B. *EMBO J.* **8**, 1403–1410 (1989).
8. Armitage, R. J. *et al. Nature* **357**, 80–82 (1992).
9. Graf, D., Korthäuer, U., Mages, H. W., Senger, G. & Kroczek, R. A. *Eur. J. Immun.* **22**, 3191–3194 (1992).
10. Hollenbaugh, D. *et al. EMBO J.* **11**, 4313–4321 (1992).
11. Spriggs, M. K. *et al. J. exp. Med.* **176**, 1543–1550 (1992).
12. Padayachee, M. *et al. Genomics* **14**, 551–553 (1992).
13. Notarangelo, L. D., Duse, M. & Ugazio, A. G. *Immunodef. Rev.* **3**, 101–122 (1992).
14. Mayer, L. *et al. New Engl. J. Med.* **314**, 409–413 (1983).