Disease models for every field

Workshop on the molecular basis of human congenital lymphocyte disorders

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The Instituto Juan March meeting on the 'Molecular basis of human congenital lymphocyte disorders' was organized by Hans D. Ochs (Seattle, WA) and José R. Regueiro (Madrid, Spain) and took place in Madrid, Spain, December 2–5, 2001. A related meeting on 'Immunodeficiencies of genetic origin' was organized by the Instituto Juan March in 1995.

expressed proteins (e.g. the DNA-dependent protein kinase, Artemis) and polypeptides confined to lymphocytes (e.g. Rag1 and Rag2). Ataxia telangiectasia and related disorders cause defects in various organs and in the immune system. Although the dysfunctional proteins responsible for these diseases are considered to be part of the same supramolecular complex, the phenotypes of the different disorders are not identical. The product of the *ATM* gene, linked to ataxia telangiectasia, is the most well-characterized of these proteins. It is involved in a variety of responses to DNA double-strand breaks, including cell cycle arrest, apoptosis and DNA repair. A.M.R. Taylor (Birmingham, UK) discussed how a DNA double-strand break repair defect might underlie the B- and T-cell abnormalities observed not only in ataxia telangiectasia, but also in Nijmegen breakage syndrome and in an ataxia telangiectasia-like disorder. The genes that are associated with these disorders, *ATM*, *NBS1* and *MRE11A*, respectively, are all involved in homologous recombination repair, and it is possible that a subtle recombination defect contributes to the immune deficiencies associated with these disorders. Heterozygous mutations of the *ATM* gene have also been implicated in a predisposition to tumour development, but the true impact remains elusive.

The ICF (Immunodeficiency, Centromeric instabilities, and Facial abnormalities) syndrome, first described in 1978, is the only inherited methylation defect in humans known to date and is due to mutations in the *DNA methyl transferase 3B* gene (*DNMT3B*). E. Viegas-Péquignot (Paris, France) reported on a second group of patients with a similar phenotype, but in which mutations are not found in the *DNMT3B* gene. It remains to be shown whether patients lacking mutations in *DNMT3A* and *DNMT3B* have a single gene defect or whether multiple genes are involved.

Cartilage-hair hypoplasia (CHH) is another monogenic human disorder affecting several tissues, including the immune system. The recently identified disease gene, *RMRP*, was found to encode a 267 nucleotide untranslated RNA molecule, which, in association with eight or nine proteins, forms an RNase MRP complex with endoribonuclease activity. CHH is due to a variety

Introduction

Primary immunodeficiencies (PID), which formerly concerned mainly geneticists, immunologists and hematologists, have now become a resource for essentially all fields in biomedicine. This is not surprising, given that the number of different forms of PID has reached more than 100 and affects such fundamental aspects of cell biology as transcription factors, vesicular transport and endoribonucleases. Table 1 lists the subsets of PID that were the focus of this meeting, subdivided according to biological function; for a more extensive description, see Ochs *et al.* (1999).

Defects of repair and modification of DNA

As discussed by A. Villa (Milan, Italy) and D. Moshous (Paris, France), several disorders of the immune system involve the DNA recombination and repair process, reflecting the role of recombination of variable, diversity and joining gene segments in the generation of antigen-specific receptors on T and B lymphocytes. This process is complex, involving both ubiquitously

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of mutations that occur either in the promoter or in transcribed regions of this gene and has been believed to be a homogeneous condition. However, I. Kaitila (Helsinki, Finland) mentioned that CHH patients without mutations in the *RMRP* gene have now been identified. He explained that these patients could have been misdiagnosed, but that it is also possible that the condition is actually heterogeneous. The latter would imply that one of the protein components of the endoribonuclease complex is mutated in these patients.

L.D. Notarangelo (Brescia, Italy) reviewed the recent developments in research on the activation-induced cytosine deaminase (*AID*) gene, which encodes the long sought-after enzyme regulating isotype switching. This gene was initially identified by T. Honjo (Kyoto, Japan), and its mutation was subsequently found to cause a human B-lymphocyte deficiency. An unexpected finding was that the corresponding protein also affects somatic hypermutation. It will be very interesting to see how this protein, which is related to RNA editing enzymes, is able to carry out both isotype switching and somatic hypermutation. Also interesting in this regard is the recent report that the Nijmegen breakage syndrome protein (this protein is related to ATM; see Table I) localizes to the class switch recombination regions and that this phenomenon is AID dependent (Petersen *et al.*, 2001).

Taken together, the data presented clearly show that although loss-of-function mutations in loci encoding different components of the DNA recombination machinery may have rather diverse phenotypic consequences, there is an intricate interplay between many of the proteins involved in these processes at the mechanistic level.

Transcription factor defects

Six transcription factors, mutant forms of which are known to cause human immunodeficiency disease, were discussed during the meeting. W. Reith (Geneva, Switzerland) described the elegant studies resulting in the identification of four unrelated transcription factors, all of which regulate the expression of major histocompatibility complex (MHC) genes (HLA class II). Interestingly, the one that serves as the master regulator—*CIITA*—seems to exert different functions at the different promoters that it controls. H.D. Ochs (Seattle, WA) discussed the forkhead transcription factor designated *FOXP3*. The corresponding gene is located on the X chromosome and causes a pleiotropic phenotype, involving several organs besides the immune system, when mutated. The *STAT1* transcription factor is a component of interferon signalling, and J.-L. Casanova (Paris, France) described his recent discovery of a mutation in the *STAT1* gene and discussed several other diseases caused by mutations in this pathway. These were found to affect IL-12, the IL-12 receptor and the IFN γ receptors. In the case of defective IFN γ signalling, patients are highly susceptible to specifically mycobacterial infections, which highlights how nature has developed rather elaborate means to combat these bacilli.

Cytoskeletal regulation and proteasome defects

The WASP protein is involved in cytoskeletal control through the interplay with Ras-like small GTPases such as Cdc42, as

discussed by Ochs. WASP displays the characteristics of many signalling molecules, namely the ability to alter its conformation following minor, post-translational modifications. Although mutations in this gene usually cause thrombocytopenia (failure in blood clotting), frequently in combination with immunodeficiency, one particular mutation—affecting a specific, single amino acid residue—leads instead to a granulocyte defect (Devriendt *et al.*, 2001).

The TAP1 and TAP2 proteins play an important role in the antigen presenting process by heterodimerizing to form an ABC transporter, called TAP. This transporter allows proteasome-generated peptides to pass through the endoplasmic reticulum membrane for loading onto MHC HLA class I molecules. Defects affecting the TAP proteins, and thereby preventing antigen presentation by HLA class I at the cell surface, cause lesions resembling Wegener's granulomatosis. V. Cerundolo (Oxford, UK) showed that activated natural killer (NK) cells are present in the granulomas formed in these patients, consistent with an inability of HLA class I molecules to switch off NK responses.

Defects in vesicular transport

Vesicle transport is a fundamental process in cell biology, delivering molecules to their required location within the cell. Although basic research in this field has provided significant insight into this process, few experiments have been as clear-cut as the examination of mutations affecting key components. G. Griffiths (Oxford, UK) discussed the effects of mutations in several genes involved in these processes. The proteins affected in the Chediak-Higashi (CHS/Lyst protein) and the Griscelli (MyoVa and Rab27a) syndromes were among those visualized using confocal time-lapse microscopy (Figure 1). Myosin Va tethered to phagosomes was found to bind to F-actin and to delay microtubule-dependent motility. Since some of these granules are also found in melanocytes, skin pigmentation defects are frequently observed in these disorders. Griffiths further reported that the loss of either Lyst or Rab27a results in impaired secretion, demonstrating the key roles that these proteins play in vesicle transport. Novel proteins critical to this pathway are also emerging from the study of other congenital disorders.

Signal transduction impairment

Protein-tyrosine kinases (PTKs) are important regulators of many processes in metazoans. The first cytoplasmic PTK gene found to be mutated in a disease was *BTK*. Lack of functional Btk protein causes an almost complete differentiation defect of B lymphocytes, designated X-linked agammaglobulinemia. C.I.E. Smith (Stockholm, Sweden) presented data on expression profiling in cell lines from such patients in an attempt to identify crucial components affected by this mutation. A total of 391 genes out of more than 12 500 analysed were found to be differentially expressed; some were of unknown function (Islam *et al.*, 2002). M.E. Conley (Memphis, TN) discussed mutations causing the phenotypically related disorder autosomal recessive agammaglobulinemia. B-cell receptor proteins and an adaptor that functions in the same pathways, BLNK, have been found to cause this disease when mutated, suggesting that multiple components of the same pathway may lead to a similar,

meeting report

Table I. Components defective in PID discussed at the meeting

Role of component	Name of component	Special feature ^a	Name of disease
DNA recombination	Rag1, Rag2	Missense mutations may cause a variant disease (Omenn syndrome)	AR ^b severe combined immunodeficiency
DNA repair	Artemis	Radiosensitivity	AR severe combined immunodeficiency
DNA repair	ATM; NBS1; MRE11	Radiosensitivity, tumours	Ataxia telangiectasia; Nijmegen breakage syndrome; and MRE11 deficiency
DNA methyl transferase	DNMT3B	Centromeric instability, facial abnormality	ICF syndrome (immunodeficiency, centromeric instabilities and facial abnormalities)
Endoribonuclease	RMRP	Pleiotropic phenotype, including malignancies	CHH
RNA editing	AID	Dual functions	Hyper-IgM syndrome, type 2
Transcription factors regulating antigen presentation	RFX5, RFXAP, RFXANK, CIITA	Regulate the same set of genes	MHC class II deficiency
Forkhead transcription factor	FOXP3	Endocrine and skin phenotype	Immune deficiency/dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX)
STAT transcription factor	STAT1	–	Inherited disorder of the IL-12–IFN γ axis (interferon signalling)
Cytoskeletal regulator	WASP	Thrombocytopenia, tumours	Wiskott Aldrich syndrome; X-linked thrombocytopenia
Proteasome-dependent protein	TAP1, TAP2	Nose and skin often affected	HLA class I deficiency
Vesicle transport	CHS protein/Lyst; myosin Va, Rab27a	Skin phenotype with partial albinism	Chediak–Higashi syndrome; Griscelli syndrome
Tyrosine kinase	Btk, Jak3, Zap70	–	X-linked agammaglobulinemia; AR severe combined immunodeficiency; combined immunodeficiency
Tyrosine phosphatase	CD45	–	AR severe combined immunodeficiency
Scaffolding protein	BLNK	–	AR agammaglobulinemia
Adaptor/displacer	SH2D1A	Fatal mononucleosis, B-cell lymphoma related to EBV infection	X-linked lymphoproliferative syndrome (Duncan disease)
Signal transducer NF κ B signalling	IKK γ	Skin phenotype, osteopetrosis, lymphoedema	X-linked anhidrotic ectodermal dysplasia with immunodeficiency
Interleukin receptors	Common γ -chain in IL-2,4,7,9,15,21R; IL-7R α	–	X-linked severe combined immunodeficiency; AR severe combined immunodeficiency
Interleukin receptor 12	IL-12R β 1	–	Inherited disorder of the IL-12–IFN γ axis
Interleukin 12	IL-12 p40	–	Inherited disorder of the IL-12–IFN γ axis
Interferon receptors	IFNR1, IFNR2	–	Inherited disorder of the IL-12–IFN γ axis
Tumour necrosis factor receptor	TNFR1	Fever	AD ^b recurrent fever, TNF receptor-associated periodic syndrome (TRAPS)
Tumour necrosis factor receptor and ligand related molecules	CD40; CD40L	Tumours are increased in CD40L deficiency	Hyper-IgM syndrome, type 3 and type 1
Apoptosis receptors	Fas	Tumours	Autoimmune lymphoproliferative syndrome
Caspases	Caspase 10	–	Autoimmune lymphoproliferative syndrome
T-cell receptor	CD3 γ , ϵ , ζ , CD8	–	T-cell receptor deficiency
B-cell receptor	IgM(μ), Ig α , λ 5	–	B-cell deficiency, AR agammaglobulinemia
Cytokines	IL-12	–	Inherited disorder of the IL-12–IFN γ axis
Anti-inflammatory protein	Cryopyrin/NALP3	Fever	AR recurrent fever, familial cold autoinflammatory syndrome, Muckle–Wells syndrome
Anti-inflammatory protein	Pyrin	Fever	Familial Mediterranean fever
Cholesterol metabolism	Melvalonate kinase	Fever	AR recurrent fever, hyperimmunoglobulinemia D with periodic fever syndrome (HIDS)

^aMost of the gene defects cause susceptibility to infections. The infections will vary according to the particular defect and are not presented in the table. For a comprehensive description, see Ochs *et al.* (1999).

^bAR, autosomal recessive; AD, autosomal dominant.

Fig. 1. Cytotoxic T lymphocytes (CTLs) kill targets by release of specialized granules. Wild-type CTLs (smaller cells, granules labelled in red with LysoTracker) destroy targets (labelled in green with an enhanced yellow fluorescent protein membrane marker) within 5 min of contact. In the absence of Lyst, killing is 10 times slower. Without Rab27a, killing is blocked.

although not identical, phenotype when defective. Jak3, which is a component of the Jak-STAT signalling mechanism, is involved in relaying intracellular responses to cytokines. W.J. Leonard (Bethesda, MD) reviewed the defects in Jak3 that cause a severe combined immunodeficiency, with a phenotype identical to that seen when the common γ -chain of the receptors for IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21 is mutated. Furthermore, he showed that selective inactivation of IL-7R α also causes severe combined immunodeficiency, demonstrating the important role of this receptor. CD45 is a membrane phosphatase expressed in hematopoietic cells and is believed to have an important regulatory function. Direct proof for this idea comes from the recent finding that mutations affecting this gene cause severe combined immunodeficiency, as reported by T. Chatila (St Louis, MO). A. Moretta (Genova, Italy) discussed the abnormal function of NK cells in the X-linked lymphoproliferative disease (XLP), an immune deficiency characterized by the inability to control Epstein-Barr virus (EBV) infections. XLP patients lack the SH2-containing adaptor/displacer protein (SH2D1A), which, in normal individuals, associates with the cytoplasmic tails of 2B4 and NTB-A, two co-receptors involved in the induction of NK-mediated cytotoxicity. In XLP-NK cells, both co-receptors mediate inhibitory rather than activating signals, resulting in the inability to kill EBV-infected targets. At present, no information is available on the function of these co-receptors in T cells from XLP patients. It cannot be excluded that altered T-cell function may contribute to the pathogenesis of the disease. In spite of the profound advancement in the NK cell field during recent years, direct evidence for a congenital PID in which a defect in NK cells is the major cause of the phenotype is still lacking.

The tumour necrosis factor (TNF) receptor family members and their corresponding ligands are trimeric molecules. Ligands are secreted or exist as transmembrane proteins in cells distinct from those expressing the receptor (TNFR). Mutations in the *TNF*

receptor 1 gene cause a recurrent fever syndrome (TRAPS), whereas rare mutations affecting the related molecule CD40, or more often its ligand, cause PID. Defective CD40 ligand, preferentially expressed by activated T cells, and defective CD40 itself, expressed by B lymphocytes, dendritic cells, endothelial and activated epithelial cells, give rise to a similar phenotype where the affected patient is highly susceptible to infections. This is a more severe phenotype than that observed in the case of defects in AID, which is uniquely expressed in germinal centre B lymphocytes. Fas (CD95) also belongs to the TNFR family, and, similarly, Fas mutations cause dominant inheritance of PID in many patients, although a recessive form is also known. The dominant inheritance is due to the trimeric origin of the receptors, only 1/8 of the receptors being composed of three functional chains when the mutant protein is expressed. However, this is not sufficient to explain the variation in disease severity observed among affected family members. Rather unexpectedly, however, F. Le Deist (Paris, France) reported that mutations in the gene encoding the Fas ligand have not been detected among more than 30 patients analysed, even though a heterozygous Fas ligand mutation has been reported in a single patient with systemic lupus. The downstream component in TNF receptor apoptosis signalling, caspase 10, also causes an autoimmune lymphoproliferative disease when defective.

Mutations affecting the antigen receptors for T and B lymphocytes were the topics of several presentations. Conley and C. Schiff (Marseilles, France) both reported that when the μ -chain of the B-cell receptor is defective, the outcome is a severe loss of B lymphocytes with accompanying agammaglobulinemia. Conversely, components of the T-cell receptor, such as the CD3 complex or the co-receptor CD8, seem less crucial for T-lymphocyte development. Consistent with these observations, loss of CD3 γ or CD3 ϵ gives a highly variable phenotype, as discussed by J.R. Regueiro (Madrid, Spain), who cautioned that gene transfer into CD3 γ -defective, mature cells might upset intrathymic regulation, whereas this would presumably not happen when targeting immature progenitors. The recent identification of mutations in the CD3 ζ -chain in the laboratory of A. Fischer (Paris, France), also reported here, will be of great interest, since loss of its associated signalling protein (Zap-70) gives rise to a severe phenotype. Whereas the γ , δ - and ϵ -chains of the CD3 complex each contain a single binding site for ZAP-70, the ζ -chain carries three sites, potentially making this entity more important for signal transduction. O. de la Calle-Martin (Barcelona, Spain) discussed mutations in the T-cell receptor accessory molecule CD8 α -chain, which give rise to highly variable phenotypes. The male first found to have mutations in this gene had severe respiratory tract infections with lung damage, whereas two affected younger sisters were healthy.

Apoptosis-related defects cause recurrent fever syndromes

M.F. McDermott (London, UK) reviewed the group of proteins that cause recurrent fever/autoinflammatory syndromes. Cryopyrin, a novel member of this group, is defective in the familial cold autoinflammatory and Muckle-Wells allelic syndromes (Hoffman *et al.*, 2001). As already mentioned, another member of this group is the TNF receptor 1, which has

been implicated in TRAPS (Table I). This family of proteins, including pyrin, is characterized by the presence of death domain-fold motifs. However, proteins lacking death domains may also cause recurrent fever syndromes when defective, as shown for mevalonate kinase, mutations in which lead to hyper-immunoglobulinemia D syndrome (HIDS). These disorders also include Familial Mediterranean fever and Crohn's disease. Familial Mediterranean fever is characterized by recurrent fever and inflammation, predominantly in the peritoneal cavity, whereas in Crohn's disease there is chronic inflammation at various sites in the gastrointestinal tract. Collectively, this implies that defects related to leukocyte apoptosis may cause diverse phenotypes. This also suggests that apoptosis genes expressed in other tissues may be involved in human disease. Possibly, the knowledge obtained from the recurrent fever syndromes could give hints regarding the mechanisms behind such defects.

Proven clinical benefit of gene therapy

Fischer discussed the recent developments in gene therapy for patients with X-linked severe combined immunodeficiency. The Paris group targeted this patient group and reported on the first successful gene therapy trial in 2000. A retroviral vector was used to ferry the common γ -chain gene into cells enriched for the CD34 early hematopoietic progenitor/stem cell marker. In the initial report, two patients were described, but now a total of nine patients have been included in the study. The first patient was treated more than 32 months ago and is still healthy; even though the T-cell levels have decreased, they are still within the normal range. Preparations are being made for the treatment of other severe combined immunodeficiencies, such as Rag deficiency, while retroviral vectors for Zap-70 deficiency are being developed, as was discussed by N. Taylor (Montpellier, France). Thus, molecular medicine is entering a new era. However, a major reason for the success in the French study was the careful selection of a disease in which the gene-corrected cells have a survival advantage. Further developments in vector design will hopefully make it possible to gain therapeutic effects even from the transfer of genes without a selective advantage. When this goal has been achieved, it will permanently change the landscape of PID research.

Concluding remarks

As more of the gene defects underlying PID are unravelled, the potential for using the corresponding phenotypes as models for other diseases is becoming apparent. In many cases, the affected gene belongs to a family of related genes. Examples include

genes encoding RNA editing enzymes (AID), caspases (Caspase 10) and transcription factors (STAT1). For these molecules, defects in related genes have either been found in humans or have even been generated by making targeted deletions in the mouse. A different scenario appears when the phenotype is not confined to the immune system but also involves other organs. Ataxia telangiectasia, the ICF syndrome and CHH represent this category. Here, knowledge of the disease mechanism from one organ system could provide insight that is also relevant for cells from other tissues.

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References

- Devriendt, K. *et al.* (2001) Constitutively activating mutation in WASP causes X-linked severe congenital neutropenia. *Nature Genet.*, **27**, 313–317.
- Hoffman, H.M., Mueller, J.L., Broide, D.H., Wanderer, A.A. and Kolodner, R.D. (2001) Mutation of a new gene encoding a putative pyrin-like protein causes familial cold autoinflammatory syndrome and Muckle-Wells syndrome. *Nature Genet.*, **29**, 301–305.
- Islam, T.C., Lindvall, J., Wennborg, A., Branden, L.J., Rabbani, H. and Smith, C.I.E. (2002) Expression profiling in transformed human B cells: influence of Btk mutations and comparison to B cell lymphomas using filter and oligonucleotide arrays. *Eur. J. Immunol.*, **32**, 982–993.
- Ochs, H.D., Smith, C.I.E. and Puck, J.M. (eds) (1999) *Primary Immunodeficiency Diseases: A Molecular and Genetic Approach*. Oxford University Press, New York, NY.
- Petersen, S. *et al.* (2001) AID is required to initiate Nbs1/ γ -H2AX focus formation and mutations at sites of class switching. *Nature*, **414**, 660–665.

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