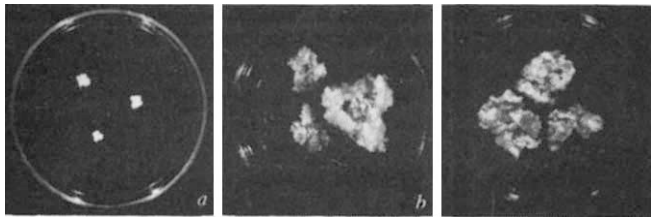

**Fig. 1** Nutritional requirement of the ILE401 line. *a*, Minimal RMOP (see Table 1 legend) medium; *b*, minimal medium with 200 mg $l^{-1}$ L-isoleucine; *c*, minimal medium with 100 mg $l^{-1}$ α-aminobutyric acid. Cultures were incubated for 3 weeks in the light (2,000 lx, 16 h).

**Fig. 2** Wild-type diploid *N. plumbaginifolia* (*a*) and ILE401 (*b*) plants. Metaphase chromosomes in root tips of an ILE401 auxotrophic plant are also shown (*c*).

Except for the first and last steps of their biosynthetic pathway, isoleucine and valine are synthesized by a common set of enzymes in microorganisms[15] and in flowering plants[16]. As isoleucine alone is sufficient to restore normal growth of the ILE401 line, it may be defective in either threonine deaminase or transaminase B (Fig. 3). There was no detectable threonine deaminase activity in crude extracts of the ILE401 line, in contrast to the wild type, in which the specific activity was $1.0 \times 10^{-3}$ μmol per min per mg protein, a value comparable with those found in other plant species[17,18]. Transaminase B activity, however, was not affected in the mutant. Specific activities in the mutant and wild type were $5.2 \times 10^{-4}$ and $3.9 \times 10^{-4}$ μmol per min per mg protein, respectively. (Details of the assays of both enzymes are given in Fig. 3 legend.) The isoleucine requirement of the ILE401 line is therefore due to the absence of threonine deaminase activity. Normal functioning of the rest of the pathway was shown in growth tests in which isoleucine could be substituted with α-aminobutyric acid (Fig. 1), which is converted *in vivo* to α-ketobutyric acid[19], the product of threonine deaminase. To avoid complications due to feedback inhibition by excess isoleucine[20], α-aminobutyric acid was used in the test, rather than α-ketobutyric acid, to provide a physiological concentration of α-ketobutyric acid (thus indirectly that of isoleucine).

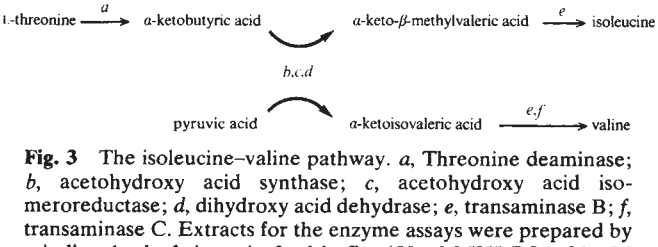


**Fig. 3** The isoleucine–valine pathway. *a*, Threonine deaminase; *b*, acetohydroxy acid synthase; *c*, acetohydroxy acid isomereroreductase; *d*, dihydroxy acid dehydrase; *e*, transaminase B; *f*, transaminase C. Extracts for the enzyme assays were prepared by grinding 1 g leaf tissue in 3 ml buffer (50 mM $KH_2PO_4$, 20 mM 2-mercaptoethanol (2-ME), 2 mM EDTA, *p*H 8.0) and centrifuging the homogenate at 15,000*g* for 10 min. Threonine deaminase activity was measured at 37 °C in the presence of 62.5 mM $KH_2PO_4$, 40 mM L-threonine, 5 mM 2-ME, 0.5 mM EDTA, *p*H 8.0. α-ketobutyric acid formed was determined by the direct method of Friedemann and Haugen[25]. Transaminase B activity was measured at 37 °C in the presence of 30 mM α-ketoglutaric acid, 25 mM L-isoleucine, 12.5 mM $KH_2PO_4$, 5 mM 2-ME, 0.5 mM EDTA and 0.1 mM pyridoxal phosphate, *p*H 8.0, using the reverse reaction. α-keto-β-methylvaleric acid formed was determined by the indirect method of Friedemann and Haugen[25].

The isoleucine requirement of the ILE401 line may be a result of a mutation or of epigenetic changes[3]. The low frequency of this phenotype, stability in culture for more than a year and expression at the plant level support the former[3]. Preliminary data on fusion with recessive mendelian pigment mutants indicate that isoleucine (and uracil) auxotrophies are recessive traits. A detailed genetic analysis of the ILE401 plants is in progress.

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# Mouse IgG3 antibodies are highly protective against infection with *Streptococcus pneumoniae*

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**Carbohydrate and protein antigens have been shown to elicit the bulk of their antibodies in mutually exclusive IgG subclasses in humans, mice, rats and horses[1–4]. In the mouse, anti-protein antibodies are primarily of the IgG1 subclass, and anti-carbohydrate antibodies are primarily of the IgG3 subclass[1,2]. We have now demonstrated that mouse IgG3 antibodies to the phosphocholine (PC) determinant of pneumococcal C-carbohydrate[5,6] and to type 3 pneumococcal polysaccharide are highly protective against experimental type 3 pneumococcal infection. This is the first demonstration that antibodies of the IgG3 subclass can protect against bacterial infection.**

Previously we have demonstrated that hybridoma IgM anti-PC antibody can protect mice from infection with type 3 *Streptococcus pneumoniae*[7] strain WU2. Other data (ref. 8 and J. B. Robbins, personal communication) indicate that hybridoma anti-PC antibody can also protect against pneumococcal types 1, 4 and 6A. Much of the anti-PC antibody produced by inbred mice expresses the variable region idiotypic marker, T-15 (ref. 9). Antibodies with this idiotype have virtually identical hypervariable region sequences[10], affinity for PC[10] and specificity for PC analogues[11]. We have used protection studies with T-15-

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positive hybridoma antibodies of different isotypes[11] to compare the effects of differences in constant regions without having to consider differences in specificity.

CBA/J mice were infected intravenously (i.v.) with $10^6$ colony-forming units (CFU) of strain WU2 type 3 *S. pneumoniae*. This dose is 10 times the $LD_{50}$ (dose that kills 50% of infected mice). Passively administered IgG3 anti-PC antibody was almost 90-fold more protective against *S. pneumoniae* on a weight basis than IgM antibody of the same idiotype (Table 1). Similarly, IgG3 anti-type 3 antibody was almost 40 times more protective than IgM anti-type 3 antibody. Protection was not observed for IgA anti-PC antibody, IgM anti-salmonella antibody, or IgG3 anti-levan antibody. Anti-type 3 antibodies were ~6–15-fold more protective by weight than anti-PC antibodies. This was expected as the PC determinants, which form part of the cell wall[6], should be largely buried beneath the type-specific capsule[12].

We repeated these studies with $(CBA/N \times DBA/2)F_1$ mice, which express the X-linked immunodeficiency *xid*[13,14], make extremely low levels of natural anti-PC antibody[7,15] and fail to produce anti-type 3 antibody when immunized[13]. These mice were infected with 150 CFU ($\sim 10 \times LD_{50}$) of *S. pneumoniae* strain WU2. IgG3 anti-PC antibodies protected *xid* mice ~10 times more effectively than IgM anti-PC antibodies, but IgG3 and IgM antibodies to the type 3 capsule showed roughly equal protective ability (Table 2). As with normal animals, anti-type 3 IgM was more effective than anti-PC IgM antibodies; however, IgG3 antibodies of the two specificities provided similar protection. The reasons for the different relative protective abilities of IgG3, IgM, anti-PC and anti-type 3 antibodies in normal and *xid* mice are unclear, but may be related to differences in immunoresponsive potential and levels of natural serum antibody of the two types of mice, or to the 4-log difference between the infectious doses used in the two studies.

**Table 1** Ability of antibodies reactive with pneumococcal cell wall and capsule to protect CBA/J mice against infection with *S. pneumoniae* strain WU2

| Hybridoma or myeloma* | Specificity | Idiotype† | Isotype† | $PD_{50}$ (μg per injection)‡ |
|---|---|---|---|---|
| 59.6C5 | PC | T-15 | IgG3 | 0.6 |
| PC-5-2 | PC | T-15 | IgM | 55 |
| T-15 and H-8 | PC | T-15 | IgA | >300 |
| 16.3 | SSS-III | ? | IgG3 | 0.1 |
| CA3-1 | SSS-III | Unique | IgM | 4 |
| CC4-6 | SSS-III | Unique | IgA | >200 |
| J606 | Levan | J606 | IgG3 | >200 |
| ST-1 | *Salmonella typhimurium* | ? | IgM | >200 |

Mice were infected i.v. with $10^6$ CFU of *S. pneumoniae*[7], and protected with intraperitoneal (i.p.) injections of 0.1 ml of the indicated immunoglobulin in doses of 0.02, 0.2, 2.0, 20 or 200 μg (or 100 or 300 μg) 1 h before infection and 1 and 2 days after infection. Injection of diluent alone (0.1% fetal calf serum) was not protective. Antibody and diluent solutions were all filter-sterilized (0.22 μm) before injection. Over 80% of deaths occurred within 4 days of infection. The experiment was discontinued after 10 days.

* Myeloma antibodies T-15, H-8 and J606 and hybridoma protein 59.6C5 were isolated by affinity chromatography[11,20,25]. Hybridoma proteins 16.3, CA3-1, CC4-6 (ref. 26), ST-1 (ref. 27) and PC-5-2 (a gift from John Kearney) were used as diluted ascites fluids. The amount of antibody in these fluids was quantitated by radioimmunoisotype assays[28]. Comparable dilutions of normal mouse serum were not protective.

† Determination of the idiotypes and isotypes has been described previously[9,11,26,28]; ? indicates unknown idiotype.

‡ $PD_{50}$, dose of passive antibody calculated[29] to protect 50% of the mice from fatal infection. At least six to eight mice were tested at each dose.

**Table 2** Ability of antibodies reactive with pneumococcal cell wall and capsule to protect $(CBA/N \times DBA/2)F_1$ male mice from *S. pneumoniae* strain WU2

| Hybridoma or myeloma | Specificity | Idiotype | Isotype | $PD_{50}$ (μg per injection) |
|---|---|---|---|---|
| 59.6C5* | PC | T-15 | IgG3 | 0.6 |
| 134.5B11* | PC | T-15 | IgG3 | 0.7 |
| PC-5-2 | PC | T-15 | IgM | 6 |
| 22.1A4[c] | PC | T-15 | IgM | 4 |
| 140.7C6.2* | PC | T-15 | IgM | 6 |
| T-15 and H-8 | PC | T-15 | IgA | >300 |
| 16.3 | SSS-III | ? | IgG3 | 0.6 |
| CA3-1 | SSS-III | Unique | IgM | 0.8 |
| J606 | Levan | J606 | IgG3 | >100 |
| ST-1 | *Salmonella typhimurium* | ? | IgM | >200 |

The mice used here express the X-linked immunodeficiency (*xid*) defect of CBA/N mice, have no naturally occurring anti-PC antibody[7,15] and are highly susceptible to pneumococcal infection. Mice were infected i.v. with 100 CFU of *S. pneumoniae* and protected as described in Table 1 legend.

* Anti-PC hybridoma antibodies[11] were produced and isolated as described in Table 1 legend.

The more efficient protection by IgG3 antibody in normal and *xid* mice is even more striking considering the fact that divalent IgG3 would be expected to have a much lower avidity than an IgM molecule for identical binding sites. IgM, which is only poorly recognized by Fc receptors[16–18], probably acts by opsonizing *S. pneumoniae*, using its highly efficient complement fixation[19]. The mechanism of IgG3-mediated killing is not so obvious. Although unaggregated IgG3 does not bind complement C1 (ref. 20), IgG3 anti-streptococcal group A carbohydrate antibody[1] can apparently lyse antigen-coated red blood cells in the presence of complement[21]. Thus the C3b receptor of phagocytes may play a part in IgG3-mediated bactericidal activity. Fc receptors for IgG3 (ref. 22) probably contribute to the anti-pneumococcal protection observed here, as they mediate phagocytosis of sheep red blood cells (SRBC) in the presence of IgG3 anti-SRBC antibody[22].

The possibility that IgG3 may mediate bactericidal activity by an antibody-dependent cellular cytotoxicity is suggested by the fact that human natural killer cells can kill bacteria[23], and the recent demonstration that passive IgG3 anti-tumour antibody can protect against tumour growth far better than IgM antibody of the same specificity[24].

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# The switch region associated with immunoglobulin $C_\mu$ genes is DNase I hypersensitive in T lymphocytes

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**It is not known whether the antigen-specific receptor of T lymphocytes is encoded by conventional immunoglobulin genes. Several T-cell lines, as well as thymus cells, have been found to contain aberrant IgM mRNAs, but other T-cell lines are apparently negative[1-4]. Rearrangement of *D* and *J* gene segments which code for portions of immunoglobulin heavy (H) chain variable (V) regions has been observed in some T cells, but no complete *V–D–J* rearrangements, as required for *H* gene expression in B cells, have been found[5]. To investigate the chromatin structure around the $C_\mu$ gene in T lymphocytes, we have probed the regions flanking the gene by mild digestion with DNase I. We report here that T-lymphocyte chromatin exhibits sites hypersensitive to DNase I in a region 5′ of $C_\mu$. In B cells, this region has been proposed to be responsible for switching to downstream $C_H$ genes.**

It has been observed in other systems that there are sites in chromatin which are hypersensitive to cleavage by DNase I[6-10]. The location of such sites can be related to the restriction map of a specific gene. It was postulated that the hypersensitivity occurs at DNA sites which may regulate gene activity in the vicinity[6-10]. To determine whether hypersensitive sites are associated with the $C_\mu$ gene in T lymphocytes, nuclei from thymus cells (>99% T lymphocytes[11]) and liver cells (as a control) were prepared and mildly treated with DNase I[12]. The range of DNase I concentrations used has been shown to digest active immunoglobulin genes without affecting inactive genes and the bulk of the DNA[12]. DNA was extracted from these nuclei and analysed by restriction enzyme digestion together with Southern filter hybridization to map DNase I-hypersensitive sites[8]. A restriction map of the $C_\mu$ gene, indicating the enzyme sites used in the analyses, is shown in Fig. 1*a*.

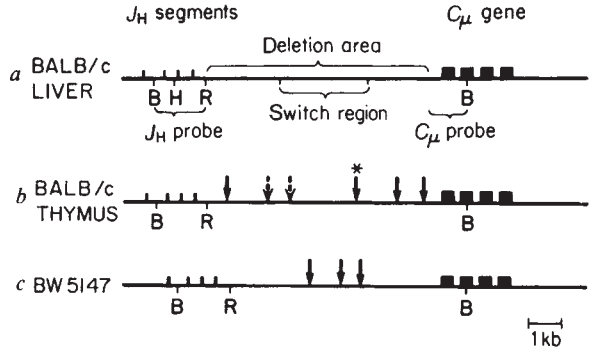


**Fig. 1** Restriction maps of the region 5′ of the $C_\mu$ gene (modified from Liu *et al.*[16]). Deletion area[15-17] and switch region[18-22] were reported for several plasmacytomas to be involved in the deletion of sequences 5′ of $C_\mu$ and the switch to other $C_H$ genes, respectively. Vertical arrows indicate DNase I-hypersensitive sites (the stippled arrows indicate sites in thymus visible with the $J_H$ probe, but indistinct with the $C_\mu$ probe); *, site that was only visible with the $C_\mu$ but not with the $J_H$ probe—this site seems also to be hypersensitive in purified DNA (see Fig. 3). The $J_H$ probe is a *Bam*HI–*Eco*RI fragment cloned by K. Marcu into pBR322 (ref. 15). The $C_\mu$ probe is a *Bam*HI–*Eco*RI (artificial *Eco* site) fragment from a germ-line library clone subcloned into pBR322 (R. Near, E. Selsing and U.S., unpublished results).

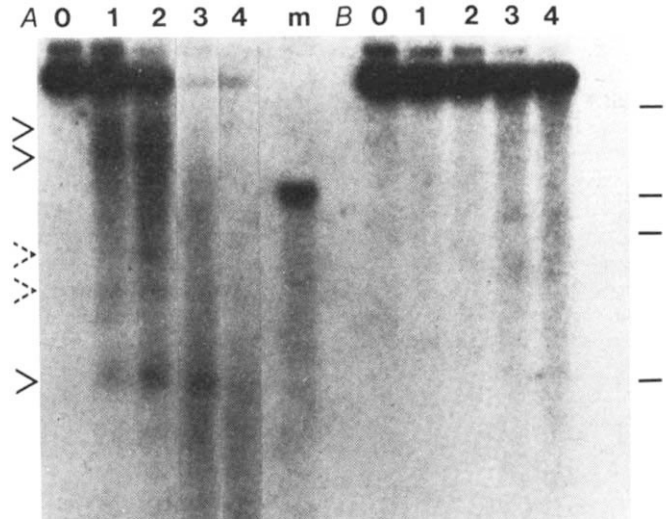


**Fig. 2** Southern blots of thymus (*A*) and liver (*B*) DNAs from untreated nuclei (track 0) and nuclei treated with DNase I (tracks 1–4) to reduce gradually the size of the DNA to an average of 15–20 kb (track 4)[12]. The DNAs were prepared and digested with *Bam*HI[13]. Southern blots[28] were produced and hybridized with a $J_H$ probe (see Fig. 1*a*). Sizing markers are indicated along the right-hand side of the gel—λ phage DNA cut with *Ava*I (8.5, 6.1, 4.8 and 2.2 kb). m, Radiolabelled sizing marker due to the hybridization of the plasmid probe with the 4.6-kb phage λ DNA fragment. Subfragments flanked by a *Bam*HI site and a DNase I-hypersensitive site are indicated by arrowheads. The major hybridization band is the $J_H$–$C_\mu$ gene; the faint fragments above it are due to incomplete digestion by *Bam*HI.

Liver cells were analysed to provide controls representing a tissue in which immunoglobulin genes are not expressed[12,13]. When liver DNA, extracted from untreated nuclei, was digested with *Bam*HI and hybridized with a particular $J_H$ probe (see Fig. 1*a*) a single restriction fragment of 9.8 kilobases (kb) was observed (Fig. 2*B*, track 0). DNAs extracted from liver nuclei treated with increasing amounts of DNase I showed the same *Bam*HI restriction fragment (Fig. 2*B*, tracks 1–4). No additional bands were observed, indicating that there are no DNase I-hypersensitive sites in this region of the liver genome.

Thymus DNA, extracted from untreated nuclei, and hybridized with the $J_H$ probe also showed the 9.8-kb *Bam*HI restriction fragment (Fig. 2*A*, track 0) seen with liver DNA. This supports the conclusion that in most thymus cells no DNA rearrangement has occurred in the region between $J_H$ and the $C_\mu$ gene. In contrast to the results obtained with liver cells, however, DNase I treatment of thymus nuclei resulted in the appearance of new fragments (indicated by arrows in Fig. 2*A*). These subfragments must be flanked by a *Bam*HI site at one end and a DNase I-hypersensitive site at the other. As the $J_H$ probe represents the 5′ end of the 9.8-kb $J_H$–$C_\mu$ *Bam*HI fragment (Fig. 1*a*), the length of the subfragments produced by DNase I digestion indicates the distance between the *Bam*HI site within the $J_H$ cluster and the DNase I-hypersensitive sites (Fig. 1*b*) thus allowing localization of the hypersensitive sites in the $J_H$–$C_\mu$ map.

We have confirmed the location of DNase I-hypersensitive sites in thymus chromatin by analysis with a $C_\mu$ probe (see

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