

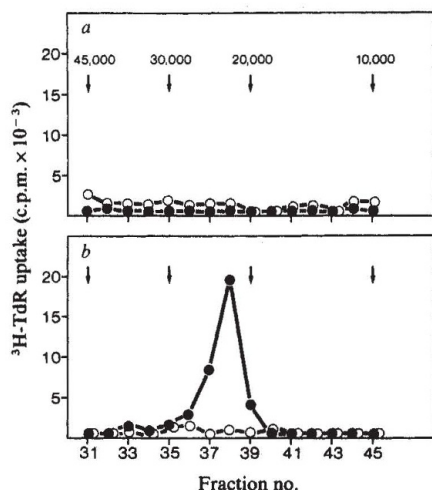


Fig. 2 Fractionation of the growth supporting activity in WEHI-3 CM. WEHI-3 CM was obtained from 5–7-d cultures of WEHI-3 cells in Iscove's modified Dulbecco's medium (IMDM) containing bovine serum albumin (BSA) ($750 \mu\text{g ml}^{-1}$), human transferrin ($30 \mu\text{g ml}^{-1}$) and soybean lipid ($50 \mu\text{g ml}^{-1}$) as described previously¹⁴. The WEHI-3 CM (760 ml) was concentrated by dialysis against polyethylene glycol (MW 20,000, Serva), applied to a Con A-Sepharose column ($2.5 \times 40 \text{ cm}$) (Pharmacia) and eluted with 50 mM Tris HCl pH 7.4 containing 0.5 M NaCl and 10^{-4} M Ca^{++} , Mg^{++} and Mn^{++} . The non-interactive material was concentrated over an Amicon Diaflo UM-10 membrane and thereafter applied to a Sephadex G-150 column ($2.5 \times 95 \text{ cm}$). Running buffer was as given in Fig. 1 legend. The growth-supporting activity was recovered in the post-albumin fractions, which were pooled and used in the experiments presented in Table 1. This pool was thereafter concentrated over a UM-10 membrane and applied to a Sephadex G-75 superfine column ($1.6 \times 90 \text{ cm}$) in the same running buffer as above. The fractions eluted from this column were tested for their capacity to support growth of *a*, T-cell blasts in the presence (●) or absence (○) of Con A ($5 \mu\text{g ml}^{-1}$); and *b*, purified resting T cells (see legend to Table 1) in the presence (●) or absence (○) of Con A ($5 \mu\text{g ml}^{-1}$). The proliferative responses were measured and each point represents the mean value of DNA-incorporated ^3H -TdR from triplicate cultures.

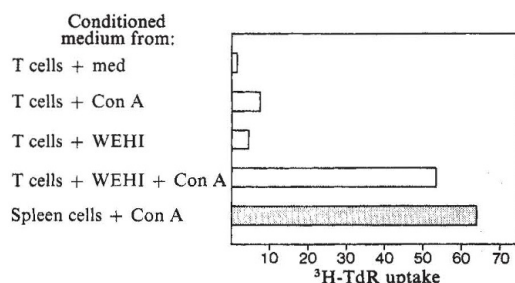


Fig. 3 WEHI-3 activity supports the Con A dependent production of TCGF by purified T cells. Spleen cells depleted of I-A-positive cells were cultured at 2.5×10^6 per ml either alone or with Con A ($5 \mu\text{g ml}^{-1}$) or partially purified WEHI-3-derived activity (pooled active fractions indicated in Fig. 2). The supernatant medium was tested for TCGF activity in T-cell blast cultures. As a positive control, normal spleen cells were cultured at 5×10^5 per ml in the presence of Con A ($5 \mu\text{g ml}^{-1}$) for 24 h. The proliferative responses were measured on day 3 of culture and the results are presented as the mean values of ^3H -TdR (c.p.m. $\times 10^{-3}$) incorporated into DNA in triplicate cultures.

T lymphocytes having some other essential role. Thus, the nature of the lectin and LAF interactions with these cells leading to the release of TCGF remain unclarified. Finally, as TCGF-dependent T-cell blasts, which include killer T cells^{5,9,17}, are not competent to product TCGF, it seems that different T-cell subpopulations are involved in production and response to TCGF. Although the requirement for TCGF by killer T lymphocytes is clear, a fuller knowledge of the requirements of the T helper cell population is needed.

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Immunisation against heterologous type II collagen induces arthritis in mice

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The induction of polyarthritis in rats by intradermal immunisation with homologous or heterologous type II collagen in complete or incomplete Freund's adjuvant was reported recently by Trentham *et al.*¹. We have now produced a similar disease in certain strains of mice.

Mice were injected with either type I or type II bovine collagen, prepared using the method of Trentham *et al.*², emulsified in complete Freund's adjuvant (CFA) or incomplete Freund's adjuvant (ICFA), in experimental conditions similar to those known to be effective in the rat. The time of onset, and incidence of arthritis were determined by daily clinical examination, regular measurement of the thickness of hind foot pads with a micrometer gauge, and after necropsy, by histological examination of proximal and distal joints in fore and hind limbs, the tail, ear and spinal column.

The findings reported here mainly concern the induction of arthritis, clinically affecting several joints, in the DBA/1 strain of inbred mice (Laboratory Animals Centre, Carshalton). Mice aged 8–15 weeks were initially injected intradermally in the back with bovine type I or type II collagen, or the acetic acid solvent for the collagens, emulsified in CFA or ICFA. Mice were challenged 3 weeks later with an intraperitoneal injection of the same type of collagen, or of acetic acid alone.

The first sign of swelling and redness of a fore or hind paw was detected after 23 d in mice injected with type II bovine collagen in CFA. The swelling progressed to a maximum at different times in individual joints and then gradually regressed, although the joints still appeared badly damaged on histological

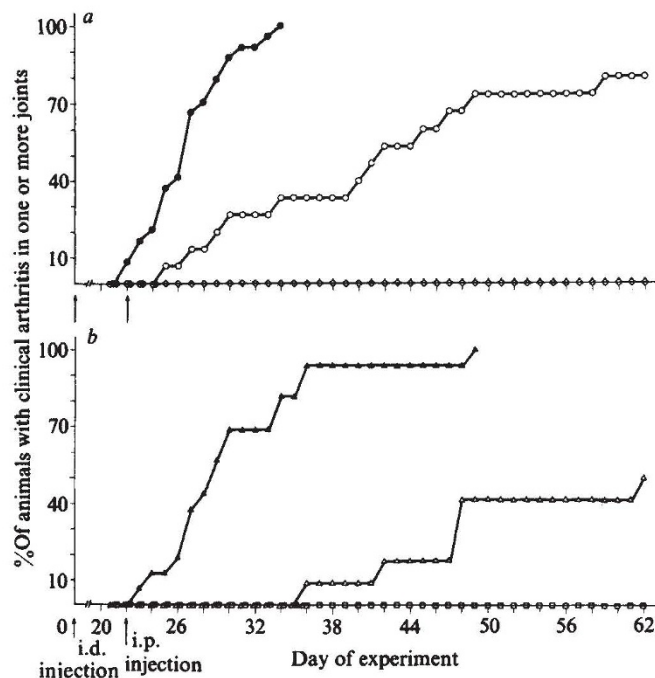


Fig. 1 Incidence and time of onset of arthritis in DBA/1 mice. DBA/1 mice were injected intradermally into 4–6 sites on the back with 100 μ g of bovine type II collagen, dissolved in 0.1 ml of 0.1M acetic acid and emulsified in an equal volume of CFA (0.5 mg ml⁻¹ *M. butyricum*; Difco) or ICFA (Difco) on day 0, followed on day 21 by an intraperitoneal injection of 100 μ g of the same collagen type dissolved in acetic acid. *a*, Onset and incidence of arthritis in male mice given type II collagen in CFA (●); type II collagen in ICFA (○); type I collagen in CFA (◇); type I collagen in ICFA (□). *b*, Onset and incidence of arthritis in female mice given type II collagen in CFA (▲); type II collagen in ICFA (△); type I collagen in CFA (◊).

examination. Several joints of a single animal were often involved, sometimes so severely that the mouse had to be killed. The onset of arthritis in female mice injected with type II collagen in ICFA was delayed—swelling was never seen before day 36 in any joint. The incidence of the disease was lower in male and female mice injected with type II collagen in ICFA (67%) than in animals receiving type II collagen in CFA (100%).

The incidence and day of onset of arthritis in mice injected in four separate experiments are shown in Fig. 1. Of 40 mice injected with type II collagen in CFA, all developed arthritis, compared with 18 of 27 injected with type II collagen in ICFA. None of the 12 mice injected with type I collagen in CFA, or of the 17 mice injected with acetic acid and CFA, showed signs of the disease (Table 1).

Histological examination of affected joints from animals receiving type II collagen either in CFA or ICFA revealed initial oedema of the synovium and perisynovial tissues, followed by synovial hyperplasia. There was an early massive, predominantly polymorph and subsequent mononuclear cell infiltration into the synovium, sub-synovium, joint cavity and periarticular tissue. This was followed by pannus development, fibrin deposition, and cartilage and bone erosion with new bone formation. Late in the course of the disease the cellular infiltration and oedema were reduced, but by then many joints had become ankylosed, and most recognisable synovium and joint cartilage had been destroyed. The vertebrae were rarely affected, involvement occurring only several weeks after initial peripheral joint swelling. Very occasional, small inflammatory foci in the ear or tail were seen in a few animals.

Several important negative observations were made. There was no clinical or histological evidence of arthritis after injection

of type I bovine collagen or acetic acid in CFA. Uninoculated control animals kept in the same cage as mice injected with type II collagen in CFA did not develop any clinical or postmortem evidence of arthritis. Synovial cavity washes of affected and unaffected joints from 17 DBA/1 mice were cultured for mycoplasma and all gave negative results. There was no significant growth of bacteria when synovial cavity washes from one unaffected and four affected animals were cultured anaerobically and aerobically, and no bacteria were seen in histological preparations of joints.

There are differences between the disease in the rat as described by Trentham *et al.*¹ and that seen in the mouse. Arthritis in the rat appeared earlier and it could be produced by immunisation with antigen in either CFA or ICFA. Mice responded less well to use of ICFA as adjuvant. A single intradermal injection has produced the disease in the rat, but only a schedule of initial intradermal immunisation followed by a subsequent challenge has been used in the mouse. We have found in both species, that involvement of second, third and fourth paws may occur up to 4 weeks after swelling of the first joint and that the number of joints affected varies between animals. The histology in the two species is similar.

Initial studies in other strains of mice treated in the same fashion showed that arthritis may sometimes be produced, but its clinical and histological incidence was variable, and the severity has been less than in the DBA/1 mice: SWR (major histocompatibility type H-2q)—4/15 (total number with clinical or histological lesions/total number treated); SJL (H-2s)—4/6, BALB/c (H-2d)—4/27; BIO.G (H-2q)—4/11; DBA/1 mice from the Jackson Laboratory, Bar Harbour, Maine—2/5 (the Carsholton colony was derived from this colony 25 years ago (M. W. Festing, personal communication); male and female mice from both colonies are histocompatible on skin grafting and *in vitro* mixed lymphocyte culture (L. Berumen, personal communication). Groups of 5–10 mice of the following strains did not develop any joint lesion after treatment with collagen type I or II in CFA or ICFA—C57B1/10ScSn (H-2b), CBA/CA (H-2k), BIO.A (5R) (H-2i5), BIOBR (H-2k) and DBA/2 (H-2d).

It is known that the immune response to the native helical portion of the bovine type II collagen molecule differs in strains of inbred mice³ and that the response appears to be under H-2 linked control⁴. Trentham *et al.*⁵ have shown that in rats the conformational antigenic site on the collagen molecule is important in induction of arthritis, since isolated α -chains of type II collagen did not produce arthritis when injected intradermally in either CFA or ICFA. Initial work has been undertaken here to study the anti-type II collagen antibody response in susceptible and resistant strains of mice, the role of the histocompatibility type and to examine the immunological processes involved.

Table 1 The incidence of arthritis induced by bovine collagen type II in DBA/1 mice

Bovine collagen type given on day 0 and on day 21		Adjuvant given on day 0	No. of mice affected by day 62/total mice given treatment
Male	II	CFA	24/24
	—	CFA	0/10
	—	—	0/5*
	II	ICFA	12/15
	—	ICFA	0/4
Female	I	CFA	0/5
	II	CFA	16/16
	—	CFA	0/7
	II	ICFA	6/12
	I	CFA	0/7

* Kept in same cage as affected animals.

The finding that an arthritis develops in mice after immunisation with type II collagen lends some support to the hypothesis of Trentham *et al.*¹ that an immune reaction to collagen may be involved in the pathogenesis of arthritis. Use of the mouse disease as a model should avoid the risk of adjuvant arthritis common to experiments in the rat, and it should also permit ready analysis of the role of histocompatibility type and immune response genes.

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Human antibody reacts with a B-cell subset in man to induce B-cell differentiation

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The events leading to B-cell differentiation and maturation into plasma cells in man are still poorly defined. Human B-cell differentiation has mainly been studied after triggering by nonspecific mitogens which react with unknown B-cell-surface structures. T cells and T-cell factors play an important part in this B-cell activation¹. It is therefore interesting that we found that some patients with the rare immunodeficiency disease, the Wiskott-Aldrich (WA) syndrome, possess an IgM antibody which reacts only with a subset of human B-cells from the peripheral blood and tonsils and which is able to induce B cells to differentiate into plasma cells *in vitro*. This triggering is largely independent of T cells and in certain conditions occurs in the absence of detectable B-cell proliferation. In view of certain recent findings in some immunologically defective strains of mice such as CBA/N, genetic immunodeficiencies in man may represent useful models for investigating human B-cell subsets and the process of B-cell activation. We have therefore looked for B-cell antibodies in the serum of WA patients because, in addition to an immune defect of poorly understood mechanism², these patients have major thrombocytopenia and are therefore multi-transfused. We found that the serum of seven of nine WA patients contained an antibody reacting by cytotoxicity and immunofluorescence tests with a subset of both normal human and, unexpectedly, of the patients' own B cells.

A two-step double labelling procedure was used for the immunofluorescence experiments. In the first step, membrane-bound B-cell immunoglobulins (Ig) were redistributed with fluoresceinated IgG or F(ab')₂ fragments from antibodies to human Ig (or μ chains), allowing the enumeration and identification of B cells. In the second step, the cells were incubated in non-capping conditions with heat-inactivated sera of the various patients or controls, washed and finally stained with rhodamine-conjugated F(ab')₂ fragments of monospecific rabbit IgG antibodies to human Ig chains³. Whereas the control sera, including 15 patients with varied immunodeficiencies, did not stain a sizeable population of B cells, the serum of seven WA

patients reacted with 30-60% of B cells from human tonsils and from the peripheral blood of all 30 normal donors tested. The antibody belongs to the IgM class and is polyclonal because the 19S (and not 7S) fraction obtained by preparative ultracentrifugation of the serum in a sucrose density gradient was reactive and, in immunofluorescence tests staining was observed only using F(ab')₂ fragments of antibodies to μ , κ and λ chains (and not to γ , α or δ). Most sera had a titre of 1/4 to 1/16 and reacted with B cells at both 4 °C and 37 °C. Two sera had an end point of 1/32 and 1/128 respectively and were also studied by cytotoxicity at 25 °C on purified B- and T-cell populations from selected donors with various HLA phenotypes. These experiments confirmed that the antibodies were unreactive with T cells and killed a subset of B cells.

The reactive antigenic determinant on the surface of B cells is independent of surface Ig (and specially IgD) as shown by double-labelling and redistribution experiments. Double labelling experiments also showed that the reactive structure is unrelated to Ia-like (or Dr) antigens, as could be expected in view of its presence on only a B-cell subset. For the same reason it seems very unlikely that the WA antibody reacts with other well defined B-cell-surface molecules such as the receptors of IgG, IgM, complement components, or Epstein-Barr virus. In addition it should be recalled that the antibody was unreactive with T cells and monocytes, as well as red cells and platelets.

The antigenic structure is presumably a marker of a unique subset of B cells and does not seem to be a differentiation antigen since we found that it is expressed on pre-B cells of a patient with pre-B-cell acute lymphoblastic leukaemia, on all leukaemic cells from two of five patients with B-derived chronic lymphocytic leukaemia and on the plasma cells from two of four myeloma cases. Experiments to define the biochemical properties and molecular weight of this antigenic structure are in progress. It is probably distinct from the glycoprotein of molecular weight 54,000 (gp 54), recognised by a rabbit antiserum and recently described by Wang *et al.*⁴, which is also a marker of a subset of human B cells, since interaction of these surface molecules with their specific antibodies mediates different functional effects.

Whereas antibodies to the gp 54 antigen stimulated B lymphocytes to proliferate but not to differentiate into plasma cells, we found that the WA antibody does induce B-cell maturation. Table 1 shows that when peripheral blood lymphocytes were cultured in the presence of 1% WA serum (or its 19S fraction at a concentration of 10 μ g ml⁻¹), a 5- to 20-fold increase in the percentage of plasma cells above control cultures was found on day 7, together with a striking cell proliferation and increase in surface Ig-positive cells. The pattern which was

Table 1 *In vitro* induction of B-cell differentiation by WA serum

		Cytoplasmic Ig*				Membrane Ig*			
		μ	γ	α	δ	μ	γ	α	
Expt 1	WA serum	12	8	5	0	23	5	8	
	NHS	1.5	0.5	<0.5	0	7	5	3	
	PWM	15	13	11	0	18	3	9	
Expt 2	WA serum	15	6	3	0	22	2	5	
	NHS	1	0.5	<0.5	0	6	4	2	
	PWM	8	12	11	0	12	2	10	
Expt 3	WA serum	14	6	7	0	21	3	6	
	NHS	0.5	1	0	0	8	4	2	

Mononuclear cells from peripheral blood were isolated by Ficoll-Hypaque gradient. The cells were washed three times in RPMI medium 1640 containing 10% FCS and antibiotics. 5×10^5 cells were incubated for 7 days in a 5% CO₂/95% air incubator in 5 ml of the same medium containing 1% WA serum, 1% normal human serum (NHS) or 50 μ g ml⁻¹ PWM. On day 7, cells were washed and studied for surface and cytoplasmic Ig by direct immunofluorescence with monospecific antisera to human Ig heavy chains.

* Percentage of positive cells.