Review article

Modulation of immune function by dietary lectins in rheumatoid arthritis

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Despite the almost universal clinical observation that inflammation of the gut is frequently associated with inflammation of the joints and vice versa, the nature of this relationship remains elusive. In the present review, we provide evidence for how the interaction of dietary lectins with enterocytes and lymphocytes may facilitate the translocation of both dietary and gut-derived pathogenic antigens to peripheral tissues, which in turn causes persistent peripheral antigenic stimulation. In genetically susceptible individuals, this antigenic stimulation may ultimately result in the expression of overt rheumatoid arthritis (RA) via molecular mimicry, a process whereby foreign peptides, similar in structure to endogenous peptides, may cause antibodies or T-lymphocytes to cross-react with both foreign and endogenous peptides and thereby break immunological tolerance. By eliminating dietary elements, particularly lectins, which adversely influence both enterocyte and lymphocyte structure and function, it is proposed that the peripheral antigenic stimulus (both pathogenic and dietary) will be reduced and thereby result in a diminution of disease symptoms in certain patients with RA.

Rheumatoid arthritis: Lectins: Immune function

Rheumatoid arthritis (RA) is a complex autoimmune disease characterized by persistent inflammation of the synovium, local destruction of bone and cartilage and a variety of systemic manifestations which may ultimately result in functional disability. Three major aspects of RA suggest a fundamental autoimmune-mediated disease: (1) the presence of often massive lymphocytic infiltrates and activated CD4+ T-lymphocytes within the inflamed synovium; (2) production of large amounts of rheumatoid factor (RF) by B-lymphocytes and plasma cells in the synovium, and (3) the observation that immuno-suppression influences the course of RA (Williams, 1996). RA affects approximately 1 % of the adult population with females being two to four times more susceptible than males (Grossman & Brahn, 1997).

Autoimmune diseases occur when the body loses the ability to discriminate self proteins from non-self proteins. This loss of tolerance ultimately results in the destruction of self tissues by the immune system. Typically, autoimmune diseases are characterized by the presence of auto-antibodies and autoreactive T-lymphocytes acting against specific self proteins (Dalton & Bennett, 1992). Most autoimmune diseases are thought to develop via the interaction of an environmental factor or factors with a specific hereditary component. The genetic components most closely associated with the expression of autoimmune diseases are those genes which code for the human leucocyte antigens (HLA). The mechanism (or mechanisms) by which autoimmunity is manifested in genetically susceptible individuals via environmental factors is not clearly defined, however, it is increasingly being recognized that the process of molecular mimicry (Fig. 1), by which a specific foreign antigen may induce an immune cross-reaction with self antigens, may be involved in a variety of autoimmune diseases (Oldstone, 1987; von Herrath & Oldstone, 1995) including RA (Albani & Carson, 1996; Baum *et al.* 1996; Wilson *et al.* 1997).

In the present review, we propose that the interaction of dietary lectins with enterocytes and lymphocytes facilitates the translocation of both dietary and gut-derived bacterial antigens to peripheral tissues, which in turn causes persistent peripheral antigenic stimulation. In genetically susceptible individuals, this antigenic stimulation may ultimately result in the expression of overt RA via molecular mimicry in which foreign peptides are similar enough in structure to cause T-lymphocytes and antibodies to cross-react with endogenous peptides and break immunological tolerance. By eliminating dietary elements, including lectins, which adversely
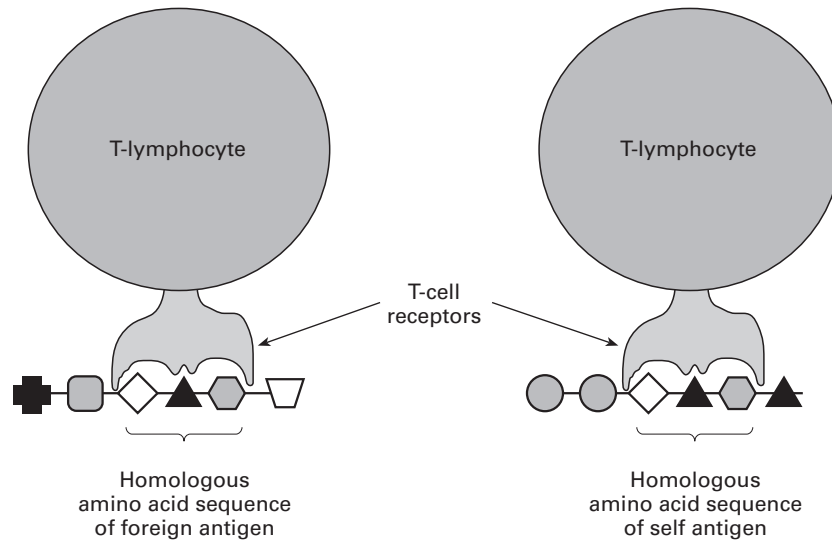

Fig. 1. A schematic representation of simple two-way molecular mimicry in which foreign and self antigens may trigger T-cell cross-reactivity because of a shared sequence of amino acids.

influence both enterocyte and lymphocyte structure and function, it is proposed that the peripheral antigenic stimulus will be reduced and thereby result in a diminution of disease symptoms.

Relationship of gut inflammation to rheumatoid arthritis

There is a strong relationship between gut inflammation and joint inflammation which has been recognized for decades in clinical practice and which has been substantiated in a variety of animal models of arthritis. Approximately 20 % of all patients with inflammatory bowel disease (Crohn's disease, ulcerative colitis) are complicated by joint inflammation (Hazenberg *et al.* 1992). Conversely, occult intestinal inflammation, which may be related to non-steroidal anti-inflammatory drug therapy or may be disease associated, occurs in approximately 67 % of patients with RA (Sartor, 1989).

Because gut inflammation is known to increase intestinal permeability, it has been suggested that an increased uptake of luminal bacterial components across the inflamed mucosa leads to a systemic distribution of bacterially derived, arthropathic products (Sartor *et al.* 1996). In support of this notion is the observation that HLA-B27 transgenic rats develop arthritis when raised in conventional environments, but do not do so under germ-free conditions (Taurog *et al.* 1994; Rath *et al.* 1996). Further, treatment with metronidazole (an antibiotic preferentially acting on anaerobic bacteria) attenuates gastrointestinal inflammation and can prevent reactivation of arthritis in animal models (Sartor *et al.* 1996). It has been consistently shown that a single intraperitoneal injection of cell-wall fragments of *Eubacterium aerofaciens*, a main resident of the human intestinal flora, can elicit both acute and chronic arthritis in a rat model (Hazenberg *et al.* 1992; Kool *et al.* 1992). The main constituents of the *Eubacterium aerofaciens* cell wall are peptidoglycan–polysaccharide complexes, of which a 65 kDa heat-shock protein is the smallest bioactive unit (Klasen *et al.* 1994). These data point to the critical role that gut-derived bacteria may play in eliciting RA.

In human subjects, as previously mentioned, intestinal inflammation frequently accompanies RA (Sartor, 1989). Further, there is considerable evidence to suggest that intestinal permeability may be increased in patients with RA (Katz & Hollander, 1989; Mielants, 1990), particularly when joint disease is active (Smith *et al.* 1985). Patients with RA have also been shown to maintain a high frequency of small-intestinal bacterial overgrowth (Henriksson *et al.* 1993), particularly with anaerobic bacterial species (Benno *et al.* 1994; Eerola *et al.* 1994). Although the mechanism of action is not entirely clear, there is convincing evidence to show that antibiotic therapy has anti-rheumatic activity in many patients with RA (Trentham & Dynesius-Trentham, 1995; O'dell *et al.* 1997). As in animal models, the human data are suggestive that increased uptake of luminal bacterial components across the inflamed mucosa leads to a systemic distribution of bacterially derived, arthropathic products.

Translocation of intestinal antigens to the periphery

Common mucosal pathogens, particularly those found in the gut, may play an important role in the aetiology of RA by virtue of their ability to initiate an autoimmune response via interaction with the immune system. Clearly implicit in this model is the ability of intraepithelial pathogens and intact proteins to escape enzymic digestion and to cross the gastrointestinal barrier and enter peripheral circulation.

From a functional perspective, in healthy subjects the contents of the gut lumen lie outside the body and contain a toxic or antigenic load from which the body needs to be protected. Protection is supplied by a number of mechanisms including: the intestinal mucosa, intestinal secretions (primarily mucus and secretory immunoglobulin (Ig)A), and intramural lymphocytes (Crissinger *et al.* 1990). The primary intestinal barrier is supported by the liver, through which all enterically derived substances must pass before entering the peripheral circulation. Under normal circumstances in healthy subjects, the intestinal immune apparatus

mounts rapid and potent effector responses to prevent invasion by pathogenic viruses and bacteria (Mowat, 1987). Further, healthy, functionally intact epithelial mucosa cells normally do not allow passage of more than small amounts (approximately 2 %) of intact dietary proteins (Mowat, 1987; Travie & Menzies, 1992). However, translocation of viable bacteria from the gastrointestinal tract to extra-intestinal sites (mesenteric lymph nodes, liver, spleen, kidney and blood) has been shown to occur under three circumstances: (1) disruption of ecological equilibrium which allows intestinal bacterial overgrowth, (2) deficiencies in host immune defences, and (3) increased permeability of the intestinal barrier (Berg, 1992).

Undegraded dietary peptides have also been shown to enter the peripheral circulation (Hurby et al. 1985), particularly when intestinal permeability is increased by disease (Travis & Menzies, 1992), non-steroidal anti-inflammatory drugs (Travis & Menzies, 1992; Bjarnason & Peters, 1996), ethanol (Bjarnason et al. 1984; Keshavarzian et al. 1994), acetic acid (Fabia et al. 1993) and dietary lectins derived from legumes and cereal grains (Liener, 1986; Pusztai, 1993).

Dietary lectins

Common dietary staples such as cereal grains and legumes contain glycoproteins called lectins which have potent anti-nutritional properties (Table 1) which influence the structure and function of both enterocytes and lymphocytes (Liener, 1986; Pusztai, 1993). Wheat-germ agglutinin derived from dietary wheat products is heat stable and resistant to digestive proteolytic breakdown in both rats (Pusztai et al. 1993a) and human subjects (Brady et al. 1978) and has been recovered intact and biologically active in human faeces (Brady et al. 1978). Wheat-germ agglutinin and lectins in general bind surface glycans on gut brush-border epithelial cells causing damage to the base of the villi which includes disarrangement of the cytoskeleton, increased endocytosis and shortening of the microvilli (Liener, 1986; Sjolander et al. 1986; Pusztai, 1993). The structural changes induced by wheat-germ agglutinin on intestinal epithelial cells elicit functional changes including increased permeability (Sjolander et al. 1984) which may facilitate the passage of undegraded dietary antigens into systemic circulation (Pusztai, 1993). High-wheat-gluten diets have been shown to induce jejunal mucosal architectural changes in normal subjects (Doherty & Barry, 1981). In rats dietary wheat-germ agglutinin is rapidly transported across the intestinal wall into the systemic circulation where it is deposited in blood and lymphatic vessel walls (Pusztai, 1993a).

Under normal circumstances, when the luminal concentration of intact dietary proteins is low, absorbed proteins generally elicit a minimal allergic response because of the limiting influence of T-suppressor cells. Because of their resistance to digestive proteolytic breakdown, the luminal concentrations of lectins can be quite high, consequently their transport through the gut wall can exceed that of other dietary antigens by several orders of magnitude (Pusztai, 1989a), and absorbed dietary lectins can be presented by macrophages to competent lymphocytes of the immune system (Hurby et al. 1985; Pusztai, 1989a).

Not only do dietary lectins increase gut permeability (Sjolander et al. 1984; Greer & Pusztai, 1985) thereby allowing increased passage of dietary and gut-derived bacterial antigens into the periphery (Liener, 1986; Pusztai, 1993), they may also cause a bacterial overgrowth which facilitates the preferential growth of gut bacteria such as Escherichia coli and Lactobacillus lactis (Banwell et al. 1988) which are associated with the expression of RA because they contain an amino acid sequence (Q(K/R)RAA) which is also found in the gene products of the HLA system of a high percentage of patients with RA (Auger & Roudier, 1997). Phytohaemagglutinin (PHA), a dietary lectin derived from kidney beans, causes accelerated enterocyte cell turnover which leads to an increase in the proportion of juvenile cells

Table 1. A non-comprehensive list of edible plants containing lectins and the physico-chemical properties of their purified lectins (modified from Liener, 1986)

| Genus and species | Common name | Toxicity | | Molecular mass (Da) | Sugar specificity |
		Peritoneal*	Oral†		
Arachis hypogaea	Peanut, groundnut	?	?	110 000	Galactose
Canavalia ensiformis	Jack bean	+	+	110 000	Mannose, glucose
Macrotyloma uniflorum	Horse gram	−	+	113 000	N-acetylgalactosamine
Lablab purpureus	Hyacinth bean	+	+	n.a.	n.a.
Glycine max	Soyabean	+	−	122 000	Galactose, N-acetylgalactosamine
Hordeum vulgare	Barley	?	+	40 000	N-acetylglucosamine
Lens culinaris	Lentil	−	?	52 000	Mannose, glucose
Lotus tetragonolobus	Winged bean	?	+	120 000	Fucose
Oryza sativa	Rice	?	?	10 000	N-acetylglucosamine
Phaseolus aureus	Mung bean	−	?	n.a.	n.a.
Phaseolus coccineus	Scarlet runner bean	?	?	120 000	N-acetylgalactosamine
Phaseolus lunatus	Lima bean	−	+	124 000	N-acetylgalactosamine
Phaseolus vulgaris	Kidney bean	+	+	120 000	N-acetylgalactosamine
Pisum sativum	Garden pea, split pea	−	−	53 000	Mannose, glucose
Ricinus communis	Castor bean	+	+	60 000	Galactose, N-acetylgalactosamine
Solanum tuberosum	Potato	?	?	46 000	Diacetylchitobiose
Triticum vulgaris	Wheat	?	+	36 000	N-acetylglucosamine
Vicia faba	Horse bean, broad bean	?	?	50 000	Mannose, glucosamine

n.a., not available.
* Death results from the intraperitoneal injection of crude seed extract or purified lectin.
† Growth inhibition caused by adding purified lectin to the diet of experimental animals.

(containing high levels of membrane polymannosylated receptor glycans) on the small-intestinal villi (Pusztai, 1993). Because *Escherichia coli* preferentially binds to mannose receptors on enterocytes, the increase in the number of mannose receptors via increased juvenile enterocytes allows *Escherichia coli* bacteria to successfully out-compete other resident gut micro-organisms. It is likely that PHA and other dietary lectins operate in the same manner to induce similar changes in other gut floral species (Pusztai *et al.* 1991).

Legume and cereal lectins alter the microflora of the gut (Liener, 1986; Banwell *et al.* 1988; Pusztai *et al.* 1993*b*), causing both inflammation (Wilson *et al.* 1980; Liener, 1986; Pusztai *et al.* 1993*b*) and increased intestinal permeability (Greer *et al.* 1985) which in turn facilitates the translocation of gut pathogens to the periphery. Kidney-bean lectin (PHA) is lethally toxic for conventional rats when given in high doses (Wilson *et al.* 1980), but is non-toxic for germ-free animals (Rattray *et al.* 1974). Thus, PHA's toxic effects could be directly attributed to its ability to increase the translocation of gut-derived bacteria to the periphery. In the case of RA, dietary lectins may operate in a similar manner to indirectly increase the expression of the disease by facilitating movement of bacterial antigens with arthrogenic properties to the periphery.

Because dietary lectins are able to cross the gastrointestinal barrier rapidly and enter the circulation intact (Pusztai *et al.* 1989), they may also be able to interact directly with synovial tissues. Although not a characteristic model of RA with all of its symptoms, a rabbit model of arthritis has shown that the direct injection of legume-derived dietary lectins into the knee joint induces the development of severe arthritis. Specifically, single injections of *Lens culinaris* lectin (derived from lentils), *Pisum sativum* lectin (derived from peas), or the lectin concanavalin A derived from the jack bean (*Canavalia ensiformis*) were able to induce severe arthritis characterized by an amplification of the initial inflammatory response due to T-lymphocyte stimulation (Brauer *et al.* 1985).

Other legume-derived lectins such as soyabean agglutinin, concanavalin A, and lectins derived from other *Phaseolus* (bean) species have been demonstrated to influence intestinal structure and function negatively (Liener, 1986), as have lectins derived from groundnuts (Ryder *et al.* 1992). Legumes are almost always consumed in the cooked state, and it is often assumed that cooking eliminates lectin activity. However, Grant *et al.* (1982) have demonstrated that residual lectin activity is present in kidney beans (*Phaseolus vulgaris*) even when cooked at 85° for 6 h or at 90° for 3 h. Lectin activity has been demonstrated in wheat, rye, barley, oats, maize (Liener, 1986) and rice (Tsuda, 1979). Maize, like wheat, can alter intestinal epithelial structure and function (Mehta *et al.* 1972). The biological activities of cereal lectins are similar because they are closely related to one another both structurally and immunologically (Peumans & Cammue, 1986).

Clinical and experimental evidence implicating diet in rheumatoid arthritis aetiology

The control of RA by dietary manipulation has been infrequently tested and has not always yielded convincing results (Shatin, 1964; Ziff, 1983; Darlington *et al.* 1986;

Buchanan *et al.* 1991), probably because the gastrointestinal tract may not play a pathogenic role in all cases of RA and because most previous clinical trials have only controlled for single, rather than multiple dietary elements which may simultaneously influence disease expression and progression.

Van de Laar & van der Korst (1992) demonstrated symptomatic improvement in a subset of patients with RA who were seropositive for RF when they were placed on elemental diets (protein-free diets consisting of essential amino acids, glucose, trace elements and vitamins). Twice as many of the food-sensitive patients showed improvement during a milk-free leg of the trial, and all food-sensitive patients showed marked disease exacerbation during food re-challenge. The authors concluded: 'The existence of a subgroup of patients in whom food intolerance influences the activity of rheumatoid factor seropositive rheumatoid arthritis deserves serious consideration'. In support of this conclusion is a more recent experiment which showed a significant ($P = 0.04$) improvement in the number of sore joints in a group (*n* 10) of patients with RA who followed an elemental diet for 3 weeks (Haugen *et al.* 1994). Further, in the only controlled study of elemental diets in the treatment of RA, patients experienced improvements in grip strength ($P = 0.008$) and Ritchie score ($P = 0.006$) that relapsed following food re-introduction (Kavanaghi *et al.* 1995). In Crohn's disease approximately 20 % of the patients experience joint inflammation together with gut inflammation (Hazenberg *et al.* 1992). Elemental diets have been shown to be as effective as corticosteroids in treating the disease, and most subjects (84 %) achieve disease remission with elemental diets (Riordan *et al.* 1993). The most frequent food intolerances were to cereals, dairy products and yeast (Riordan *et al.* 1993).

In coeliac disease there is a characteristic T-cell-mediated destruction of the intestinal villi which results in malabsorption and increased intestinal permeability (Hamilton *et al.* 1982). All symptoms of coeliac disease are eliminated following removal of gluten-containing cereals (wheat, rye, barley and oats). RA has frequently been demonstrated to occur concurrently with coeliac disease (Collins & Maki, 1994; Lepore *et al.* 1996). Multiple studies of arthritic patients have demonstrated elevated antibody levels for gliadin (O'Farrelly *et al.* 1988; Lepore *et al.* 1993), and gluten-free diets have been shown to be effective in reducing arthritic symptoms in coeliac patients (Bourne *et al.* 1985; Charkravarty & Scott, 1992; Lepore *et al.* 1993). These studies support the concept that wheat-containing diets can increase intestinal permeability and thereby allow gut-derived antigens access to the periphery. Because removal of gluten-containing grains not only eliminates coeliac disease, but also symptoms of arthritis, such diets may be of benefit for some patients with RA. No large clinical trials have been undertaken specifically to examine the effectiveness of gluten-free diets in the treatment of RA, however, there are numerous case studies reporting alleviation of RA symptoms with grain-free diets (Shatin, 1964; Williams, 1981; Beri *et al.* 1988; Lunardi *et al.* 1988). Additionally, complete withdrawal of food during fasting reduces objective and subjective indices of the disease (Kjeldsen-Kragh *et al.* 1991). Collectively, these studies suggest that modulation of intestinal physiology by dietary substances may allow both

dietary and pathogenic antigens access to the periphery, thereby causing persistent immune system stimulation.

Milk and dairy products have frequently been implicated in the aetiology of RA. O'Farrelly *et al.* (1989) demonstrated that fifty-three of ninety-three patients with RA had elevated circulating IgG antibodies to milk, wheat or both dietary proteins. Bovine serum albumin (BSA), a milk protein, contains an amino acid sequence homologous with human collagen type I, C1q, and sera from RA patients displayed reactivity to synthetic peptides containing the BSA residues responsible for the homology (Perez-Maceda *et al.* 1991). Additionally, exogenous BSA peptides have been found to be bound to RA HLA-DR susceptibility alleles (Chicz *et al.* 1993). Case studies have shown that elimination of milk and dairy products from the diets of patients with RA improved symptoms, and the disease was markedly exacerbated on re-challenge (Parke & Hughes, 1981; Panush *et al.* 1986). No large-scale controlled trial testing the effect of dairy products on RA development and progression has been undertaken. In animal models of RA, disease symptoms are routinely induced in dogs (Ohashi *et al.* 1996), rats (Griffiths, 1992) and rabbits (Thomsen *et al.* 1985) by injecting the synovium with BSA. Further, milk drinking is known to induce rheumatoid-like joint lesions in rabbits drinking cows' milk (Welsh *et al.* 1985).

Immunological and molecular mechanisms of rheumatoid arthritis

Genetic susceptibility

Inherited susceptibility to RA is associated with the genes found on the short arm of chromosome 6 which code for the HLA system. On chromosome 6, the HLA system is sub-divided into class I (HLA-A, HLA-B, HLA-C) and class II segments (HLA-DR, HLA-DQ, HL-DP). Within the class II segment, the HLA-DRB1 genes, which encode the HLA-DR4 and HLA-DR1 molecules, convey enhanced susceptibility to RA. The specific function of HLA molecules is to bind internally processed antigens (both exogenous and endogenous in nature) and to present them to T-lymphocytes. Thus, the relationship between HLA susceptibility haplotypes and RA indicates an antigen-driven response (Weyland & Goronzy, 1997). More specifically, it has been observed that most (76%) of the HLA alleles associated with RA contain, in the third hypervariable region of their β chains, an amino acid sequence composed of the amino acid motif Q(K/R)RAA (glutamine-(lysine/arginine)-arginine-alanine-alanine) (Rowley *et al.* 1997). Not only does the Q(K/R)RAA motif increase the probability of RA, it increases the severity of destruction (Larsen score > 1·62) and the likelihood of developing early erosive disease (Wagner *et al.* 1997). How the Q(K/R)RAA motif increases RA susceptibility or severity is still controversial. However, a multistep molecular mimicry model (Fig. 2) has been proposed where the Q(K/R)RAA sequence is an antigenic epitope exhibited on several gut microbial proteins (*Escherichia coli*, *Lactobacillus lactis*, *Brucella ovis*, *Proteus mirabilis*), and patients with RA respond more strongly to these antigens than healthy subjects (Albani & Carson, 1996; La Cava *et al.* 1997; Auger & Roudier, 1997).

Molecular mimicry

HLA molecules themselves are frequently processed and presented by antigen-presenting cells and these HLA-derived peptides sometimes represent the majority of
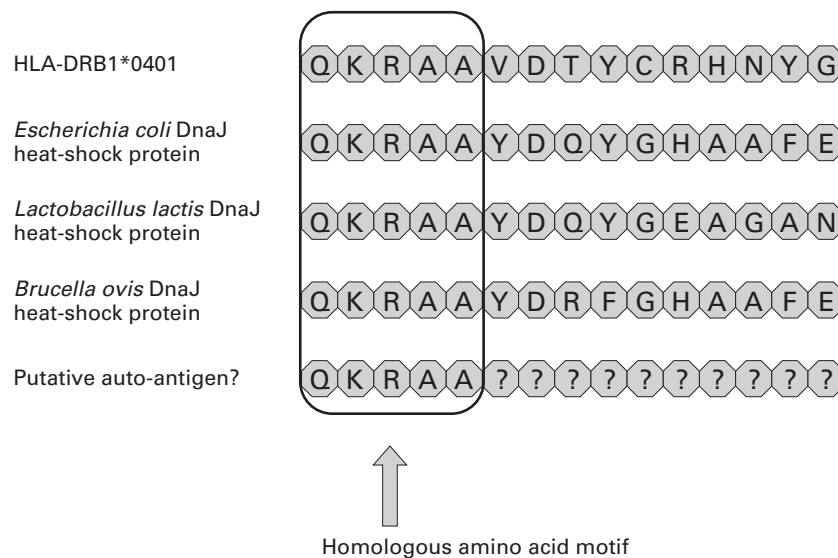

Fig. 2. Rheumatoid arthritis may arise from three-way molecular mimicry in which the Q(K/R)RAA (glutamine-lysine/arginine-arginine-alanine-alanine) amino acid susceptibility motif is shared by gut bacterial proteins, by self human leucocyte antigen (HLA) proteins and by a putative tissue auto-antigen. Bacterial peptides containing the Q(K/R)RAA sequence and entering the periphery (facilitated by dietary lectins) may stimulate T-cells causing them to cross-react with the putative auto-antigen.

peptides presented by the antigen-presenting cells (Cao *et al.* 1995). It has been suggested that thymically selected T-cells with weak affinity for self HLA peptides may subsequently be stimulated by peripheral exposure to microbial peptides which mimic the HLA amino acid sequence (Baum & Staines, 1997). Specifically, the T-cell repertoire which is positively selected during embryonic development, with weak affinity for the Q(K/R)RAA motif expressed on HLA-DR molecules, may subsequently be stimulated by peripheral exposure to *Escherichia coli*, *Lactobacillus lactis*, *Brucella ovis* and *Proteus mirabilis* containing the mimicking epitope. The Q(K/R)RAA motif, whether expressed on HLA-DR molecules, synthetic peptides, bacterial or viral proteins represents a strong epitope for B-lymphocytes responsible for serological cross-reactivity among Q(K/R)RAA-containing peptides (Roudier *et al.* 1989; Albani *et al.* 1992).

The Q(K/R)RAA motif is also the basis for T-lymphocytes involved in the positive and negative selection of the T-cell repertoire in the thymus during embryonic development. The T-lymphocyte repertoire in most cases is entirely defined before exposure to environmental pathogens (Bevan *et al.* 1994), and immature T-lymphocytes are positively selected in the thymus by virtue of their low-affinity binding with HLA peptides presented by cells of the thymic epithelium. These positively selected T-lymphocytes undergo maturation and become part of the pool of mature, naive T-lymphocytes that, after birth, are recruited in specific immune responses (Jameson *et al.* 1995). Immature T-lymphocytes in the thymus that bind with high affinity to HLA self peptide complexes have a high self-reactive potential and are eliminated (negative selection) (Nossal, 1994). Therefore, thymically selected T-lymphocytes which have been positively selected by virtue of their low affinity interactions with HLA-DR1B alleles containing the Q(K/R)RAA motif, may be later triggered in the periphery on exposure to foreign peptides with homologous amino acid sequences (Albani & Carson, 1996).

Rheumatoid factor

Approximately 70–80 % of patients with RA have RF present in their blood and synovial fluid (Grossman & Brahn, 1997). RF is an autoantibody since it has specificity for the Fc (C γ 3 and C γ 2 domains) receptor of IgG (Williams, 1992). Recent work utilizing crystal structure analysis of the RF–IgG Fc, antibody–antigen complex suggests that RF may have another entirely different specificity separate from IgG Fc and that the reactivity with IgG Fc probably occurs because of similarities with an unidentified antigen (Sutton *et al.* 1998). Consistent with this notion is earlier work indicating that viral and bacterial proteins also bind IgG Fc receptors via microbial receptors which resemble the IgG Fc receptor (Nardella *et al.* 1985, 1988). Further, RF Ig genes show clear evidence of somatic mutation, indicating that RF production by B-lymphocytes is a T-lymphocyte-dependent, antigen-driven process (Goronzy & Weyland, 1993). More recent studies utilizing computer modelling and crystallographic studies suggest that the mechanisms that operate on RF selection in RA synovia are similar to immune responses to exogenous antigens (Mageed *et al.* 1997). Collectively, these data suggest that RF production may occur principally in response to foreign proteins and secondarily in response to self proteins.

There is some evidence to suggest that RF production may be influenced by dietary proteins. O'Farrelly *et al.* (1989) showed that fifty-three of ninety-three patients with RA had raised levels of IgG antibodies to milk and/or wheat proteins. Of the fifty-three patients positive for dietary proteins, forty-eight (90 %) had raised levels of IgA RF whereas only seven (17 %) of the remaining forty non-diet-sensitive RA patients had detectable levels of IgA RF. These data are suggestive of a breakdown in gastrointestinal tolerance to dietary antigens in this group of patients, and indicate that RF production may occur in response to gastrointestinally related antigens.

Interaction of dietary lectins with immune function

It is apparent that dietary lectins from both cereal grains and legumes increase the translocation of gut-derived bacterial and dietary antigens to the periphery by: (1) causing an intestinal bacterial overgrowth and (2) increasing intestinal permeability. Additionally, dietary lectins have the ability to interact with components of the immune system which may facilitate the autoimmune process. Table 2 lists these

Table 2. Influence of dietary lectins on gastrointestinal and immunological function

Effect	Reference
1. Facilitate preferential growth of bacteria such as *Escherichia coli* and *Lactobacillus lactis* which contain the Q(K/R)RAA susceptibility motif	Liener, 1986; Banwell *et al.* 1988; Pusztai *et al.* 1993*b*
2. Bind surface glycans on gut brush-border epithelial cells causing disarrangement of the cytoskeleton, increased endocytosis and shortening of the microvilli	Liener, 1986; Sjolander *et al.* 1986; Pusztai, 1993
3. Increase gut permeability allowing increased passage of both dietary and bacterial antigens to the periphery	Sjolander *et al.* 1984; Greer & Pusztai, 1985; Liener, 1986
4. Amplify HLA class II expression in intestinal epithelial cell lines	Weetman *et al.* 1985
5. Stimulate T-cell proliferation	Uder *et al.* 1980; Clevers *et al.* 1986
6. Stimulate IFN-γ, causing HLA class II expression	Piccinini *et al.* 1987; Lowes *et al.* 1992
7. Cause abnormal expression of ICAM in T-cells	Koch *et al.* 1994; Shingu *et al.* 1994
8. Stimulate the production of inflammatory cytokines (IL-1, TNFα)	Firestein *et al.* 1990; van den Bourne *et al.* 1997

HLA, human leucocyte antigens, IFN-γ, interferon-γ; ICAM, intracellular adhesion molecules; IL-1, interleukin-1; TNFα, tumour necrosis factor α.

multiple effects, and Fig. 3 shows schematically how dietary lectins may facilitate the expression of RA via their interaction with the gut and the immune system.

Dietary lectins including both wheat-germ agglutinin (Pusztai *et al.* 1993*a*) and PHA (Pusztai *et al.* 1989) have been shown to cross the gastrointestinal barrier rapidly and enter the peripheral circulation. In rats dosed with PHA, up to 10 % of the lectin is found in circulation 3 h after feeding (Pusztai *et al.* 1989). Thus, PHA is a powerful oral immunogen which produces a high titre of IgG anti-PHA antibodies in animals and probably man (Pusztai, 1993). Antibody development to PHA becomes measurable 10 d after the first dose and further feeding or re-introduction results in booster effects (Pusztai, 1993). Thus, the gut anti-lectin IgA system is ineffective against PHA since it cannot prevent its absorption after its re-introduction (Pusztai, 1989*b*). This abrogation of the gut IgA response to PHA and possibly to other lectins has important consequences in autoimmune disease because it would allow dietary lectins continuous access to T-lymphocytes at the gut mucosal surface.

Further, because dietary lectins increase intestinal permeability, they would promote increased activity between the immune system and gut bacteria. The interaction of the systemic immune system with bacterial and dietary antigens at the gut mucosal surface could lead to activation of previously quiescent Q(K/R)RAA-specific T-lymphocytes which react with the Q(K/R)RAA amino acid motif of gut pathogens (Albani & Carson, 1996). Normally, these activated T-lymphocytes would return to the intestinal mucosa and would not travel to peripheral sites, such as the synovium, without abnormal expression of intracellular adhesion molecules (Albani & Carson, 1996). Numerous *in vitro* experiments have demonstrated that PHA is a potent stimulator of intracellular adhesion molecule expression in RA (Koch *et al.* 1994; Shingu *et al.* 1994). Thus, lectin-induced intracellular adhesion molecule expression in autoreactive T-lymphocytes would allow them to travel to peripheral sites in the joint and to persist in the synovial membrane.

Immunogenic foreign antigen fragments could be brought to the inflammatory sites by synovial type A macrophages and by B-cells recruited and activated by the inflammatory stimuli (Albani & Carson, 1996). Therefore RF-producing B-cells, because of their ability to bind and ingest antigens trapped in immune complexes, would represent powerful
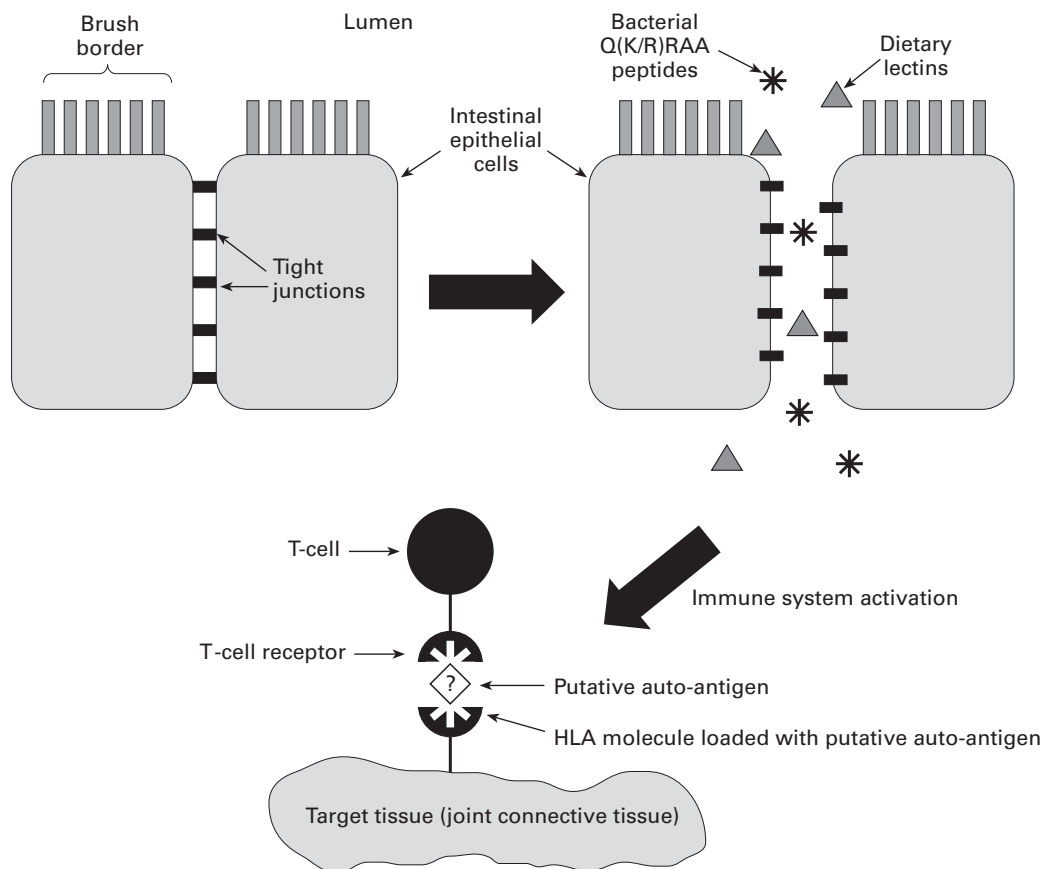

Fig. 3. A diagrammatic illustration of how dietary lectins may hypothetically interact with the gut and immune system to influence the expression of rheumatoid arthritis. Dietary lectins may: (1) facilitate preferential growth of bacteria such as *Escherichia coli* and *Lactobacillus lactis* which contain the Q(K/R)RAA susceptibility motif, (2) increase gut permeability allowing increased passage of both dietary and bacterial antigens to the periphery, (3) amplify human leucocyte antigen (HLA) class II expression in intestinal epithelial cell lines, (4) stimulate T-cell proliferation, (5) stimulate interferon-γ, (6) cause abnormal expression of intracellular adhesion molecules in T-cells, and (7) stimulate the production of inflammatory cytokines (interleukin-1, tumour necrosis factor α).

antigen-presenting cells, and their presence would partially regulate the amplification of the inflammatory process (Albani & Carson, 1996).

In addition to maintaining elevated levels of intracellular adhesion molecules, RA is also characterized by elevated levels of the inflammatory cytokines, interleukin 1, and tumour necrosis factor α (Odeh, 1997). Numerous *in vitro* experiments show that PHA is a potent stimulator of both cytokines in peripheral blood mononuclear cells (Firestein *et al*. 1990; van den Bourne *et al*. 1997). To date no trials have been conducted to determine if *in vivo* administration of dietary lectins in human subjects is able to elicit similar responses. However, such responses seem likely since intact PHA is present in the peripheral circulation following its ingestion (Pusztai *et al*. 1989).

Molecular mimicry of dietary antigens with self proteins

We have outlined the hypothesis that the homologous amino acid motifs among bacterial antigens, the HLA-DRB1 genes and putative auto-antigens in joint tissue may induce RA in genetically susceptible individuals by virtue of immunological cross-reactivity in a three-way model of molecular mimicry. In addition to bacterial antigens, viral antigens, including the Epstein-Barr virus, may also induce cross-reactivity in RA via three-way molecular mimicry (Albani & Carson, 1996; Baum *et al*. 1996). Less well appreciated are the homologous amino acid motifs which may occur between dietary peptides and self and which have been implicated in the aetiology of RA.

Perez-Maceda *et al*. (1991) showed that the sera from patients with RA recognized BSA from cows' milk and that a sequence of BSA (residues 141–157) was highly homologous with human collagen type I and the plasma complement protein, C1q. In support of the immunogenicity of this BSA fragment, sera from patients with RA displayed a specific reactivity for a synthetic peptide containing the BSA residues responsible for the homology (Perez-Maceda *et al*. 1991). Chicz *et al*. (1993) have demonstrated that exogenous BSA proteins can be bound to various HLA alleles, including DR4 molecules, suggesting that BSA proteins may have a significant role in the development of autoimmunity via molecular mimicry.

Ostenstad *et al*. (1995) have demonstrated that glycine-rich cell-wall protein (GRP 1.8), which is a ubiquitous storage protein found in virtually all cereal grains and legumes, contains significant amino acid homologies with both fibrillar collagen, procollagen and Epstein-Barr virus nuclear antigen-1. A synthetic fifteen amino acid sequence derived from GRP 1.8 stimulated T-lymphocytes taken from the synovial fluid of patients with RA and caused a preferential expansion of synovial T-cells bearing V α 2.1/V β 5.5 gene products, thereby indicating the involvement of HLA complex-restricted auto-antigen recognition (Ostenstad *et al*. 1995). The T-cell expansion caused by the GRP 1.8 analogue could be blocked by the addition of anti-DR antibodies (Ostenstad *et al*. 1995), providing further evidence of the potentiating role of molecular mimicry in the aetiology of RA.

A third dietary antigen which may also induce RA via molecular mimicry is the α-gliadin component of wheat which shares significant amino acid sequences with calreticulin, an endoplasmic reticulin chaperone protein (Karska *et al*. 1995). Anti-calreticulin antibodies have been found in patients with RA (Routsias *et al*. 1993), and HLA-DR4 molecules from arthritic patients are known to present a peptide fragment derived from calreticulin (Verreck *et al*. 1995).

In summary, dietary peptide fragments, derived from both milk proteins and cereal grain and legume proteins, maintain significant amino acid homologies with collagenous tissues found in the synovium and are capable of stimulating T-cells in an HLA restricted manner. Because of the inherent lectin-induced permeability and floral changes induced by cereal and legume consumption on the intestinal epithelial cells, dietary antigens (with molecular mimicking potential) which normally would not enter into the systemic circulation, are rendered capable of doing so.

Summary

We have provided extensive evidence linking dietary substances to the development of RA. Dietary glycoproteins, as well as other elements, can influence intestinal structure and function so as to allow increased translocation of both pathogenic and dietary antigens to the periphery causing persistent immunological stimulation. Because of shared amino acid motifs among exogenous peptides, HLA-derived peptides and self tissue, cross reactivity may occur thereby breaking immunological tolerance and resulting in the expression of RA. It is proposed that by eliminating certain dietary elements, including lectins, which adversely influence both enterocyte and lymphocyte structure and function, the peripheral antigenic stimulus will be reduced and thereby result in a diminution of disease symptoms in some but not all patients with RA.

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