4.0 Discussion

4.1 Natural Antibodies against Adrenal Hormones and Other Steroid Hormones

The term naturally occurring antibodies (NA) refers to antibodies that are generated with no apparent antigenic stimulus. An example of natural antibodies is blood group antibodies. The idiotypic network theory offers an explanation for the occurrence of these antibodies in normal serum. For example, a report by Mudarris & Peck (1987) showed the natural occurrence of antibody to estrogen receptor in all serum samples tested. According to his work the antibodies do not appear to be species specific and is present in human, rat, and mouse sera and reacts equally well with receptors from human, calf, and rat uteri.

According to the findings of Stefansson *et al* (1985) and Daar & Fabre (1981), various autoantibodies are normally present in the blood of healthy individuals. Guilbert *et al* (1982) postulated that NHS contains natural antibodies to a wide spectrum of self-antigens.

Marchalonis *et al* (1993) reported that clinically healthy humans as well as patients suffering from various autoimmune diseases produce natural antibodies against a variety of self-components. Such antibodies have been proposed to carry out a physiological role in maintaining the integrity of self, as well as potentially destructive roles in the generation of autoimmune diseases. He reported that human autoantigens, are usually present in extremely small amounts, and because of this it is generally difficult to obtain

enough to carry out a detailed characterization of the antibodies or the antigenic determinants recognized.

Exposure early in life to foreign antigens with a structural resemblance to an as yet unexpressed internal antigen such as a steroid receptor could lead to the production of antibody in response to the foreign antigen that is reactive with the receptor.

Hormones are released from specific secretory cells in one part of the body, dispersed in extracellular fluid (usually blood plasma), and interact with target cells elsewhere. The binding by hormone receptors and presumably natural antihormone immunoglobulins (Igs) both bind hormones with high affinity and specificity. This similarity presumably reflects structural similarities, as evidenced by the ability of antiidiotypic antibodies, raised against the active site of antihormone Igs, to interact with the corresponding hormone receptors.

Regulatory Natural Antibody in Blood Pressure Control?

Hormones play a role in cardiovascular diseases. Some of the cortical hormones (hydrocortisone, aldosterone, androstenedione), medullary hormones (adrenaline, noradrenaline) and some other related hormones (β-estradiol, angiotensin II, acetylcholine) are involved in cardiovascular diseases, and directly or indirectly related with blood pressure control.

In this study, we have found natural antibody to aldosterone, noradrenaline and hydrocortisone. These hormones are important in volume dependent and vascular-

dependent mechanisms in arterial pressure respectively. The binding by natural antibody may function to regulate their biologic activity.

Neurohumoral, renal, metabolic, race, genetic, and environmental factors have been shown to be related to the development of hypertension; thus, the multifactorial hypothesis of the pathophysiology of hypertension was given by Randall (1991). Most of the morbid events due to hypertension and other risk factors are related to alterations of the large arteries of the brain, the heart, or the kidney. Large arteries are not passive conduit tubes but are characterized by elastic properties and are able to synthesize many vasoactive substances. These properties make the arterial wall a major modulator of the blood pressure and more generally of the cardiovascular regulation (Lajemi *et al*, 1999).

A few studies have demonstrated that adrenal antibodies may be present in the normal population and in patients with organ-specific autoimmune diseases without clinical signs of adrenal failure (Sherbaum & Berg, 1982; Betterle *et al* 1983; Leisti *et al* 1983; Ketchum *et al* 1984; Ahonen *et al* 1987). According to Sherbaum & Berg (1982), adrenal antibodies in healthy individuals are extremely rare, the prevalence being below 1/1000 and according to Spinner *et al* (1969) and Nerup (1974) adrenal autoantibodies have been seldom detectable in normal individuals. These studies usually employ immunohistology of adrenal tissue sections. Earlier, Salim *et al* (1988) reported that ELISA test could not detect antibodies (IgG) to hydrocortisone in normal person. In contrast, we now show that in a sensitive ELISA system, natural antibodies are commonly detected to adrenal hormones.

In this study NAA were found to bind hydrocortisone, and aldosterone. The structures of these hormones are shown in Appendix 3. The three isotypes of antibody (IgA, IgG, and IgM) were detected. Both IgG and IgM showed significant binding with hydrocortisone and aldosterone. IgA showed a 100% binding with aldosterone, but negative binding with hydrocortisone (Table 4.1).

The differential binding of the different isotypes to these blood pressures related hormone needs to be further studied (See electronic letter, Appendix 11).

Table 4.1 : Natural Antibody Binding with Hormones

Hormones	IgA	IgG	IgM
Hydrocortisone	–	+	+
Androstenedione	–	–	–
Aldosterone	+	+	+
Adrenaline	+	+	–
Noradrenaline	+	+	+
Progesterone	+	+	+
Hydroxyprogeste.	+	–	–
Testosterone	+	+	+
β - Estradiol	–	+	+
Angiotensin II	–	–	–
Acetylcholine	–	–	–

+ : Positive binding - : Negative binding

Steroid hormones have to form a complex with specific hormone receptors in the cytoplasm of cells before they can interact with their main target, the cell nucleus, mainly the DNA. One suggested action of natural antibody is that these autoantibodies to corticosteroid hormones may inhibit the passage of the hormones into the cell cytoplasm and hence render the hormone inactive.

Whether an imbalance i.e. an excess of such autoantibodies in disease state would lead to a peripheral insufficiency(Glascow et al (1985) and Green et al (1984) remains to be determined.

Davies *et al* (1999) reported a significant correlation of body sodium and potassium with blood pressure which might suggest a role for aldosterone in essential hypertension. In patients with this disease, the ratio of plasma renin to plasma aldosterone may be lower than in control subjects and plasma aldosterone levels may be more sensitive to angiotensin II infusion.

Natural antibodies against medullary hormones have also been tested (Table 4.1). NAA were found to bind adrenaline and noradrenaline. Adrenaline was strongly reactive with IgA, least reactive with IgG and completely negative with IgM. Noradrenaline showed a positive binding with all the isotypes of antibody.

The preferential binding to noradrenaline compared with adrenaline is also of interest and needs to be further addressed.

Catecholamines, which circulate mainly free in the plasma, are comparatively easily excreted, or attacked by enzyme in the blood and tissues. These hormones therefore tend to remain in the blood stream for only brief periods - minutes to a few hours.

The sympathetic nervous system participates in the regulation of carbohydrate, lipid, and energy metabolism, and has been implicated in the pathogenesis of hypertension and obesity. Increased sympathetic nervous system activity with age may alter disease risk and contribute to the development of certain chronic diseases. Dvorak & Poehiman (1998) reported that plasma noradrenaline appearance rate is a minor contributor to variation in mean arterial blood pressure in older, normotensive women. In the light of the presence of natural antibody to noradrenaline it would be of interest to analyze for any age-dependent pattern in the antibody.

Recently, Pogozheva *et al* (1998) also showed the existence of natural antibodies against adrenaline and noradrenaline in patients with cardiovascular disease. We observed that in this study, no NAA were found to bind angiotensin II and acetylcholine (Table 4.1). In contrast, Rozanova *et al* (1998) reported the presence of natural antibodies against Angiotensin II in blood sera of patients with cardiovascular disease.

Natural Antibodies to Hormone Receptors

In this study (Table 4.1) we found for the first time, NAA that bind some other related hormones. Progesterone and testosterone showed highly positive results with all the antibody isotypes, but hydroxyprogesterone showed positive result with IgA and negative results with IgG and IgM. β-Estradiol showed negative results with IgA but positive binding with IgG and IgM.

The significance of these natural antibodies in normal reproductive physiology remains speculative at present.

Besides NAA to hormone that are described here, NAA to hormone receptor have also been reported. Weigel *et al* (1981) reported antibodies against progesterone receptors in sheep, discovered that one third of the animals had endogenous Ig activity, which reacted with the chick progesterone receptor.

The first evidence of an interaction between estrogen receptor (ER) and IgG was reported by Jungblut & Jensen (1966). He found that after many attempts to purify ER by adsorption to matrix-bound estradiol yields a product that was contaminated with IgG. Peck (1980) detected naturally occurring human antibodies against the estrogen receptor. He investigated the nature of the serum factors and found that the serum-complexing activity was an IgG that was present in human serum. According to Mudarris & Peck (1987), ER antibody was present in virtually every serum (262 individuals ranging in age from 1-85 yrs) (tested), regardless of the individuals age, sex or reproductive status.

4.2 Epitopes of Apo E Precursor Recognized by Antibody in Normal Human Serum

The antigenic reactivity of proteins resides in restricted parts of the molecule known as antigenic determinants or epitopes. It interacts specifically with circulating (humoral) antibodies. Three types of antigenic sites have been defined: sequential, continuous, and discontinuous. Sequential determinants are those in which the antibodies recognize the linear sequence of amino acids. Such determinants are rare in globular proteins but can be found in fibrous proteins (Crumpton, 1974). A continuous antigenic site is a conformational distinct portion of the protein that is comprised of amino acids in continuous peptide bond linkage. A discontinuous antigenic sites a conformational distinct portion of the protein that is made up of amino acids not in continuous peptide bond linkage. Such sites arise from the secondary and tertiary folding of proteins. (Shinnik *et al*, 1983).

In this study peptides of the complete Apo E precursor were synthesized. Apo E is a protein of known sequence and is a component of several classes of plasma lipoproteins. Epitopes were identified by synthesizing peptides and testing these in ELISA with NHS, cord blood sera, cerebrospinal fluid, atherosclerosis patients sera and with purified IgG. On testing the synthesizing peptides with NHS some peptides showed higher optical density values, thus higher antibody binding than the other peptides. The results of this Apo E epitope study are also compared with the results from other studies.

4.2.1 Epitope Sites of Different Antibody Isotype

In this study, results showed that different antibody isotypes bound to different epitopes of Apo E with a few exceptions. Table 4.2 showed that natural IgA bound to five Apo E epitopes, IgG bound to seven epitopes while IgM bound to six epitopes.

The differences may be due to the different binding capacity of antibody isotypes. In NHS, IgG is always in monomer form while IgA and IgM exist in different forms. This difference may account for the different binding capacity. Another difference may be the percentage of these isotypes in serum or plasma. When the amount of antibody is high, this may increase its probability to bind to epitopes. Thus, in this case, IgG and IgM showed more epitopes in comparison with IgA. Avrameas (1991) reported that most of the NAA in adult sera is of the IgG class.

Some epitope similarities were observed with different antibody isotypes (Table 4.3). Some of the antibody isotypes bound to the same epitopes of Apo E.

Table 4.2 Epitope of Apo E Recognized by Different Antibody Isotypes in Normal Human Serum

Antibody	Peptides
IgA	6, 23, 24, 35, 41
IgG	1, 2, 8, 23, 33, 34, 35
IgM	6, 15, 22, 32, 33,41

Table 4.3: Antibody Isotypes in NHS Bound to Common Epitopes of Apo E Precursor

Antibody	Peptides
IgA, IgG	23, 35
IgA, IgM	41
IgG, IgM	33

Different affinity of antibody to the epitopes may influence the binding capacity. An antibody may have a higher affinity to bind to some particular epitopes due to the amino acid sequence and configuration. Geysen *et al* (1987) and Tribbick *et al* (1989) reported that several factors are implicated for a successful peptide-antibody binding. Binding involves principally the complementarity between antigen combining site of antibody, and is maintained at least partially in both shape and charge. Binding requires molecular geometry. Stereochemistry of residues in binding peptides, direction of the main chain and adequate peptide length are other contributing factors to substantial antigen-antibody binding.

We observed that the binding of NHS to apo E peptides in ELISA tested with IgA showed lower binding values than the other antibody isotype. I have tested 5 sera of NHS. The concentration of horseradish peroxidases conjugated rabbit anti-human IgA was first set at 1:3000 dilution. With this concentration the final ELISA values were very low. To resolve this problem, we increased the concentration of secondary antibody to 1:1000 dilution. Out of 4 different sera tested using this dilution we observed that 2 of the sera showed higher binding values than the serum tested with secondary antibody at 1:3000 dilution. 2 of the sera showed no distinct difference between the dilutions. Thus if the effect of increasing concentration of antibody on the binding profile is to be observed, the same sera should be used for dilution.

4.2.2 The Epitopes of Apo E and its Described Functions

Apo E plays a major role in the metabolism of plasma lipoproteins. Functions of Apo E regions are shown in Table 4. 4. The results of natural antibodies binding to these regions are also indicated. The detailed epitope sequence associated with each apo E function are shown below. Apo E is involved primarily in lipid transport and clearance by mediating the binding of lipid particles to specific lipoprotein receptors in cells (Mahley, 1988).

Table 4.4: Functions of Apo E Regions and Epitopes of Natural Antibodies

Functions	IgA	IgG	Purified IgG	IgM
Interact with lipoproteins receptor (AA 137 - 149, 144 - 156)	2	1	1	None
Interact with lipid binding (AA 207 - 219, 221 - 233, 263 - 275)	2	2	1	2
Interact with various proteoglycans, including heparin (AA 142 - 147)	1	1	1	None
Interact with β–amyloid binding (AA 200 - 299, 129 - 169)	4	4	3	4

(The numbers in the table indicates the number of epitopes found in this study)

This table shows the presence of natural antibody epitope within the different functional regions of apolipoprotein E.

Apo E consists of three distinct structural domains, a 22 kDa amino terminal domain (1 - 194), a 12 kDa carboxyl-terminal domain (216 - 299), and a 35 amino acid random structural domain. The amino-terminal domain contains a stable globular structure containing the sequence that mediates low density lipoprotein receptor binding (Wilson *et al* (1991), and the carboxyl terminal domain is less stable and contains a lipid binding site (Mahley, 1988).

Rall *et al* (1982) reported that the primary and secondary structures of Apo E reveal that there are specific regions responsible for the two known functions of Apo E, i.e.,

* Specific cell receptor binding
* Lipid binding

Human plasma Apo E is a ligand for the low-density lipoprotein receptors. It targets cholesterol-rich lipoproteins to LDL receptors on both hepatic and peripheral cells. It has been recognized for many years that the binding of Apo E to the LDL receptor is dependent on its association with lipid (Innerarity *et al*, 1979). Apo E devoid of phospholipids had only very little or no LDL receptor binding activity, which could be restored upon addition of dimyristoylphosphatidylcholine (Innerarity *et al*, 1984).

LDL Receptor Binding

It has been demonstrated by Dyer *et al* (1995) that the region of Apo E responsible for its binding to the LDL receptor has been localized to amino acids 140 - 160. Similar findings were reported by Dyer *et al* (1991) and Raffai *et al* (1995). According to Dyer & Curtiss (1991) the amino acid residues 141 - 155 of Apo E are

Table 4.5: Predicted Amphipathic Epitopes of apo E and Natural Antibody Binding

Epitopes reported by Stanley *et al*	Epitopes found in this study	Antibody Isotypes
Residues 203 - 221	207 – 219	IgG, IgM, Purified IgG
Residues 226 - 243	221 – 233	IgA, IgG
Residues 245 - 266	-	-
Residues 268 - 285	263 – 275	IgA, IgM

Some similarities were observed between the helical regions of apo E found by Rall *et al* and the epitope in this study.

This may suggest that NAA can influence the apo E interaction with lipids by interacting with the same lipid-binding sites.

Heparin Binding

Apo E epitopes involved in heparin binding have been proposed by Weisgraber *et al* (1986). The presence and identification of heparin binding sites on Apo E were established with Apo E monoclonal antibodies (Table 4.6).

Residues 142-147 are contained within the epitope for the Apo E monoclonal antibody 1D7. This heparin binding site is expressed when Apo E is complexed with lipid. The 3H1 antibody, the epitope of which, is located in the carboxyl-terminal region of the protein (residues 243-272), inhibits heparin binding. For 6C5 antibody, the epitope is located in the amino-terminal region of the protein. It has been discussed that heparin binding sites of Apo E contribute to heparin binding in the lipid-free protein (Weisgraber *et al*, 1986). Thus, in the absence of lipid, heparin binding sites on Apo E are capable of binding to heparin.

Table 4.6: Epitopes of Apolipoprotein E involved in Heparin Binding

Antibody	Epitope	Effect on Apo E binding to heparin
1D7	Vicinity of residues 140-150	Inhibits
6C5	Partially or completely contained within residues 1-13	Inhibits
3H1	Residues 243-272	Inhibits

Reference: Weisgraber *et al*, 1986

In this study, we found NAA with residues 142-147 with IgA, IgG, and purified IgG (Table 4.7). Apo E is normally associated with lipid on the surface of circulating plasma lipoproteins. When Apo E is complexed to phospholipid or on the surface of lipoprotein particles, the binding capacity is higher. This suggests that residues 142-147 may be the in vivo epitope recognized by natural antibody.

Therefore, besides LDL receptor and lipid binding, natural antibody could presumably also modulate the interaction with heparin.

β-Amyloid Binding

The Apo E has been proposed to bind amyloid precursor and change its metabolic fate or to enhance aggregation of amyloid protein (Wisniewski *et al*, 1994). In human amyloid deposits, Apo E is usually present as degraded form.

Apo E epitopes involved in beta-amyloid peptide binding activity have been proposed by Pillot *et al* (1999) which included residue 200 - 299. They demonstrated that the carboxyl-terminal lipid-binding domain of Apo E (e.g. residues 200 - 299) is responsible for the beta-amyloid peptide binding (Abeta) activity of Apo E and that this interaction involves pairs of Apo E amphipathic α - helices. In this study, we found some similar epitopes of natural antibody within this range (residues 200 - 299) (Table 4.7). *Thus this suggest that epitopes recognized by natural antibody may have a role in regulating beta-amyloid peptide binding activity.*

Table 4.7: Apolipoprotein E Regions Involved In β -Amyloid Binding and Natural Antibody Epitopes

Epitopes recognized by Pillot *et al*, 1999	Epitopes found in this Study	Antibody
Residues 200 - 299 (Carboxyl-terminal)	221 - 233, 263 - 275	IgA
	207 - 219, 214 - 226, 221 - 233	IgG
	207 - 219, 214 - 226	Purified IgG
	200 - 212, 207 - 219, 263 - 275	IgM
Residues 129 - 169 (Amino-terminal)	137 - 149, 144 - 156	IgA
	137 - 149	IgG
	137 - 149	Purified IgG
	130 - 142	IgM

The epitopes that are essential for Abeta binding are located at the carboxyl-terminal. On the contrary, Pillot *et al* (1999) reported that the amino-terminal receptor binding domain of Apo E (e.g. residues 129 - 169) is not able to form stable complexes with Abeta (Table 4.7). Strittmatter *et al* (1993) reported that residues 244 - 272 in the carboxyl-terminal domain of Apo E are presumed to be the region binding to Abeta. In this study, we found some similar epitopes but the number of epitopes found in amino region is less than the carboxyl region.

The epitopes of the monoclonal antibodies (YK-2 Murine hybridoma) was demonstrated by the Toshiyuki *et al* (1997) at the residues 221 - 233 of Apo E. In this study, we found epitopes at residues 221 - 233 with IgA and IgG. Aleshkov *et al* (1999) reported that the presence of at least one cysteine contributes to the efficient Abeta binding. It has also been documented that the affinity binding of Abeta to Apo E is greatly influenced by the conformational state, which differs in its abilities to form amyloid (Aizawa *et al*, 1997). LaDu *et al* (1994) reported that the several factors may affect Apo E behavior, such as its state of oxidation, lipid association, the presence of other amyloid, Apo E binding protein, and the stage of the amyloid process.

We found that most of the epitopes were located at the middle and carboxyl region of the Apo E molecule (Table 4.8). The middle region ranged from amino acids 130-226 and carboxyl end is ranged from 221-275. According to Marcel *et al* (1991), the middle region is mobile and accessible with multiple epitopes that are short, discontinuous and limited to a single helix, then forming a β - turn with all or part of an adjacent α - helix. Rall *et al* (1982) reported that the carboxyl-terminal portion of Apo E is predicted to contain several α- helical structures with amphipathic character and appears to be one of

the major lipid binding region of Apo E. As mentioned, this suggest that epitopes found in this study may have a NAA against the lipid binding region of Apo E.

Theoretically, when NAA bind to different epitopes of Apo E that are involved in particular functions, that function may be inhibited or blocked. This may be one of the homeostasis pathways whereby NAA participate in the negative feedback mechanism. For example, when excessive activation of LDL receptor has occurred, NAA might bind to the epitope sites that are involved in LDL receptor activation to inhibit the continuous activation of LDL receptor.

4.3 Epitopes of Apolipoprotein E Recognized by Antibody in Atherosclerosis Patients Sera

Atherosclerosis is a disease that can affect people at any age, although it usually doesn't pose a threat until people reach their forties or fifties. It is characterized by a narrowing of the arteries caused by cholesterol-rich plaques of immune-system cells. Atherosclerotic coronary artery disease is associated with the gene encoding apolipoprotein E, a ligand for the LDL receptor. It is also associated with immune activation. Key risk factors for atherosclerosis, which can be genetic and/or environmental, include: elevated levels of cholesterol and triglyceride in the blood, high blood pressure, cigarette smoke, diabetes, having a personal or family history of heart disease, cardiovascular disease, peripheral vascular disease, high blood pressure and kidney disease involving dialysis. Defects in apolipoprotein E sometimes result in its inability to bind to the receptors, which leads to an increase persons blood cholesterol, and consequently their risk of atherosclerosis.

Antibodies against oxidized low-density lipoproteins (ox-LDL) have been proposed to be independent predictors of atherosclerosis development. Recent studies by Hulthe *et al* (1998) indicate that the relationship between the autoimmune response to ox-LDL and the extent of atherosclerosis is more complex then previously anticipated. According to Holvoet and Collen (1998) work, increased levels of autoimmune antibodies against oxidatively modified LDL and increased levels of oxidized LDL antigen appear to be associated with atherosclerotic cardiovascular disease. Recent data from different laboratories have provided evidence that the first stages of atherosclerosis

are inflammatory in nature. Metzler *et al* (1998) suggested that the role of immune system in atherogenesis could enhance the mechanism of vascular disorder leading to atherosclerosis.

Although the above findings have shown the presence of autoantibodies against lipoproteins in atherosclerosis patient's sera, however no epitope mapping has been done for Apo E in atherosclerosis patient's sera. In this study, the autoantibodies in atherosclerosis patients sera were found to bind to the synthetic peptides of Apo E. IgG showed strong binding in atherosclerosis patient's sera. Table 4.9 shows that IgA bound to five Apo E epitopes, IgG bound to ten epitopes, while IgM bound to seven Apo E epitopes. The differences may be due to the different binding capacities of antibody isotypes. Most of the epitopes observed in this study are located at the middle and carboxyl region of Apo E molecule.

Compared with epitopes of apo E bound by natural antibodies in normal human sera, the atherosclerotic sera also recognized some unique apo E epitopes (Figure 4.1). Whether reaction of the patients antibodies with these apo E epitopes contribute to the atherosclerotic process needs to be evaluated further.

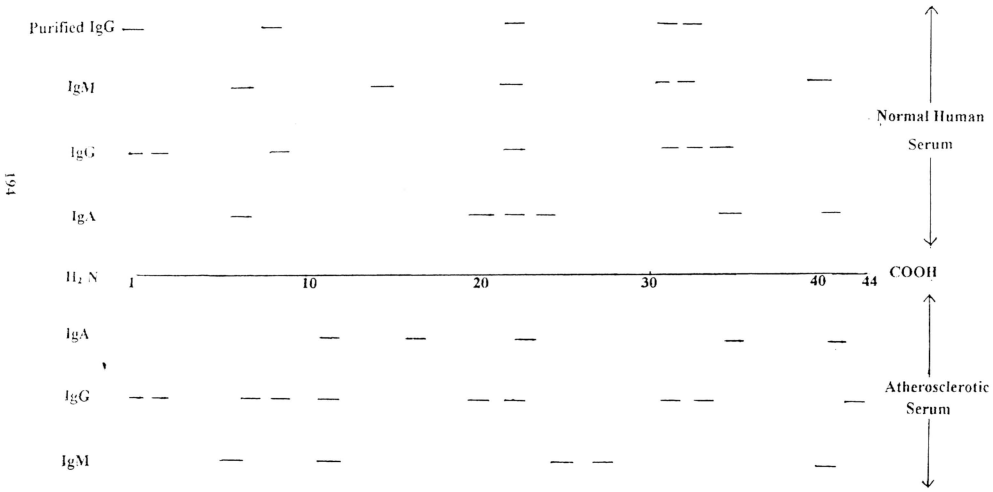

Figure 4.1: Epitope of apo E recognised by antibody in normal and atherosclerotic sera

Table 4.9: Epitopes of Apo E Detected by Antibodies in Atherosclerosis Patient's Sera

Antibody	Epitope	Amino Acid Sequence	Amino Acid residue in Apo E precursor	Amino Acid residue in Apo E
IgA	11	SSQVTQELRALMD	71 - 83	53 - 65
	16	ETRARLSKELQAA	106 - 118	88 - 100
	23	LASHLRKLRKRLL	155 - 167	137 - 149
	35	MGSRTRDRLDEVK	239 - 251	221 - 233
	41	SWFEPLVEDMQRQ	281 - 293	263 - 275
IgG	1	MKVLWAALLVTFL	1 - 13	0
	2	LLVTFLAGCQAKV	8 -20	0
	7	RWELALGRFWDYL	43 - 50	25 - 37
	8	RFWDYLRWYQTLS	50 - 62	32 - 44
	11	SSQVTQELRALMD	71 - 83	53 - 65
	20	VQYRGEVQAMLGQ	134 - 146	116 - 128
	22	TEELLVRLASHLR	148 - 160	130 - 142
	33	AQAWGERLRARME	225 - 237	207 - 219
	34	LRARMEEMGSRTR	232 - 244	214 - 226
	43	AGLVEKVQAAVGT	295 - 307	277 - 289
IgM	6	TEWQSGQRWELAL	36 - 48	18 - 30
	11	SSQVTQELRALMD	71 - 83	53 - 65
	25	DADDLQKRLAVYQ	169 - 181	151 - 163
	27	GAIEGAERGLSAI	183 - 195	165 - 177
	28	RGLSAIRERLGPL	190 - 202	172 - 184
	41	SWFEPLVEDMQRQ	281 - 293	263 - 275
	43	AGLVEKVQAAVGT	295 - 307	277 - 289

(Apo E precursor has 317 amino acids while Apo E has 299 amino acids. The first 18 amino acids at amino end are cleaved from the precursor to generate Apo E)

Oxidation of low-density lipoprotein is considered to be the initial and crucial step in the development and progression of atherosclerotic process. Autoantibodies to oxidized LDL have been detected in human serum. Bui *et al* (1996) used an ELISA technique to measure autoantibody titers in patients with coronary artery disease. They reported that the significantly higher titers of autoantibodies to ox-LDL were seen in patients with angiographic evidence of coronary artery disease compared to NHS. These findings are slightly similar with the findings of Maggi *et al* (1994). According to Maggi *et al* (1994), oxidation of LDL in coronary artery disease patients are due to high level of total plasma cholesterol, LDL cholesterol and triglycerides, and a lower level of HDL cholesterol. These are all major factors involve in oxidation of LDL.

Recent findings by O'Brien *et al* (1996) hypothesize that not only oxidised lipid components of LDL leads to atherosclerosis, but oxidative modification of proteins particularly cell-associated proteins are also a causal factor.

Whether such oxidation might affect the epitopes recognized by antibody in normal and atherosclerotic sera remains to be determined.

In this study, the presence of antibody binding shows that some of the functions which are involved in progression of atherosclerosis disease can presumably be regulated by antibody in atherosclerosis patient's sera. Antibody-related events might then lead to some disturbances in lipid and protein metabolism. In contrast, natural antibodies to ox-LDL may help to attenuate the pathophysiologic activity of the modified lipoproteins (Cheng & Sundram, 1998).