not increase plasma activities of these enzymes except by causing hepatic injury. We did, however, include drug-induced injury in the differential diagnosis of both acute and chronic liver injury and emphasized the importance of taking a drug history. We agree with the authors that this is an important consideration.

The issue of whether to include both AST and ALT in “standardized liver tests panels” was of considerable interest to our group. Our position, as stated in the guidelines, is that both enzymes, along with several other tests, should be included in a “hepatic panel” (1, 2). We agree, however, that there are cases where a complete hepatic panel is not needed. For example, we did not recommend use of AST or ALT for monitoring patients with acute hepatitis once they have begun to decrease. We also stated that ALT is the most important test in screening for chronic hepatic injury.

There is considerable evidence, however, that AST and ALT together are of use in the differential diagnosis of acute hepatic injury and in monitoring patients with chronic hepatic injury. In acute hepatic injury, AST typically is increased to a greater degree than ALT in the very acute stages of injury, as with acetaminophen or ischemic liver injury. Recognition of this ratio often provides a clue to the presence of one of these two etiologies. As pointed out by Larsson and Tryding and in our guidelines, a high ratio is typical of alcoholic hepatitis. Although they suggest that modern assays with pyridoxal-5’-phosphate may not show such a ratio, the article by Matloff et al. (3) showed that correction of plasma pyridoxine deficiency did not abolish the high AST/ALT ratio in alcoholic hepatitis. In unpublished studies of patients with hepatitis C, we have consistently seen a much higher AST/ALT ratio in persons with both alcohol abuse and hepatitis C than in those with hepatitis C alone, and in 7% of samples, only AST is increased. Furthermore, as indicated in our guidelines, an increasing AST/ALT ratio is relatively specific for development of cirrhosis in patients with chronic hepatitis C infection, and may be present long before clinical symptoms of decompensation develop. Thus, we believe that use of both AST and ALT is indicated in monitoring persons with chronic hepatic injury.

We do agree with the authors that “routine” use of both AST and ALT in screening tests is excessive. We applaud their intervention efforts in reducing AST ordering after consultation with clinical colleagues. For screening purposes, as our guidelines indicate, ALT is a superior test and can usually be used alone. In patients with known liver disease, we believe that use of both tests provides additional clinical information that is worth the small additional cost.

References

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Significance of Autoantibodies to Oxidatively Modified LDL in Plasma of Children with Down Syndrome

To the Editor:

Oxidative modification of blood lipoproteins is an important risk factor in the development of some pathological states such as atherosclerosis (1, 2). One of the sources of oxidatively modified lipoproteins in blood is the interaction of native lipoproteins with active oxygen species generated by activated neutrophils and monocyte-macrophages. In vitro studies have suggested that modification of LDL by lipid peroxide products is one potential mechanism (3–5). Modification of LDL by malondialdehyde (MDA) or other lipid peroxides in vivo is a prerequisite to the formation of arterial foam cells (6), and the presence of antibodies to lipid oxidation products suggests that oxidatively modified LDL (ox-LDL) is expressed in the artery wall (7). In vitro treatment with MDA can induce the expression of specific epitopes on oxLDL (8).

In the general population, antibodies to oxLDL and lipoperoxidations in plasma are correlated with a high risk of premature atherosclerosis. Individuals with Down syndrome (DS) show signs of premature aging, and several authors have proposed the DS population as an “atheroma-free model” (9, 10). Opinions differ as to which lipid or lipoprotein is the most important in predicting the development of atherosclerosis. Most studies compared subjects with DS with matched individuals admitted to the same institution for other disabling disorders or with unselected healthy controls. Simon et al. (11) found high serum cholesterol in young DS patients, but subsequent studies have not confirmed this in groups of affected individuals ranging in age from 6 to 60 years (12). Triglyceride concentrations have been reported to be decreased (10), increased (13), or unchanged (9) in patients with trisomy 21 compared with matched controls.

We studied two groups of children: 15 apparently healthy controls (8 males, 7 females; mean age, 4 years; range, 3–5 years) and 40 children with trisomy 21 (20 males, 20 females; mean age, 4.5 years; range, 2–7 years). We determined MDA by the LPO-586 assay (Oxis International), which is based on the reaction of a chromogenic reagent (10.3 mmol/L N-methyl-2-phenylindole in acetonitrile) with MDA at 45 °C (14). One molecule of MDA reacts with two molecules of the reagent to yield a stable chromophore with maximal absorbance at 586 nm. For the measurement of oxLDL antibodies in plasma, we used an ELISA (GULL; Design International, Kennebunk, ME) with purified oxLDL bound to
ELISA plate wells. The colorimetric end-point is read at 405 nm. The concentration of anti-oxLDL IgG is proportional to oxLDL absorbance (15).

The DS population had high concentrations of MDA (mean ± SD, 2.97 ± 1.59 μmol/L compared with 1.41 ± 0.62 μmol/L for controls; P <0.025). In addition, we found high concentrations of anti-oxLDL antibodies (52.12 ± 18.47 activity units/mL) in DS patients, even very young ones, compared with controls (29.21 ± 5.13; P <0.000025). The chemically reactive lipids (MDA) released during lipid peroxidation convert LDL, the major carrier of plasma cholesterol, to an abnormal form, and receptor-mediated clearance of this altered LDL produces cholesterol ester deposition in macrophage-derived foam cells of atheroma. In patients with atherosclerosis, this LDL content is increased (12). A growing body of evidence suggests that oxidative modification of LDL enhances its atherogenicity. Oxidative modification converts LDL to a form recognized by the macrophage acetyl-LDL receptor. During lipid peroxidation a variety of highly reactive aldehyde products are generated that, in turn, can form covalent bonds with protein, principally lysine residues. MDA is one of these products and readily reacts with lysine residues. MDA-LDL in the vesel wall could be the immunogen giving rise to autoantibodies in both aortic lesions and healthy aortic walls (13).

oxLDL induces an activation-related signal, which modulates the expression of growth factors, adhesion molecules, and tissue factors that stimulate vascular smooth-muscle cell proliferation and monocyte and T-cell migration. T cells are present in early atherosclerotic lesions and may constitute up to 20% of cells in the fibrous cap of advanced human lesions, but their role in atherogenesis is largely unknown (16). T-cell clones recognizing oxLDL in the presence of monococytes have been established from atherosclerotic plaques (17), and oxLDL may induce humoral immunity, as shown by the presence of oxLDL antibodies in hypercholesterolemic rabbits or atherosclerotic patients (18).

The discrepancy between the low incidence of atherosclerosis in DS patients (10) and the high risk associated with their increased lipoperoxidation and anti-oxLDL antibodies is apparent from our data and the results obtained by Bakalova et al. (19). The chronic heart disease affecting >40% of DS patients is presumably congenital (20) and unrelated to lipid oxidation. The Sh3bgp gene, recently isolated and mapped to chromosome 21 within the DS congenital heart disease minimal region (21), is expressed in the earliest stages of mouse heart development. It may yet turn out to play a role in heart morphogenesis and consequently in the pathogenesis of congenital heart disease in DS patients.

The OX-LDL reagent set was kindly supplied by Bouty SpA (Milan, Italy), and the LPO-586 reagent set was kindly supplied by Prodotti Gianni (Milan, Italy). We are grateful to E. De Simone (Hospital Cardarelli, Naples, Italy) for the control samples and to Judy Baggett for revising the language.

References

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To the Editor:

We conducted two studies of serum antibodies against oxidized LDL (IgGoxLDL) in mothers and their infants during the past 2 years. We used an ELISA (oLab; Eli-Tec Laborreagenzien GmbH) based on the use of Cu²⁺-oxidized LDL particles bound to the surface of microtiter plate wells and an anti-human IgG-bound to the surface of microtiter plates. In the first study, we were unable to extract IgGoxLDL, whereas others had values below those in newborns. The mean value was 815 ± 469 units/L compared with 337 ± 214 units/L in newborns. At that time, we were unable to explain these findings.

One year later, we repeated the study and obtained similar results. All mothers were familiar with the aim of the study and gave informed consent. Of the fourteen 3-month-old infants in the study, 8 had low serum IgGoxLDL, but 6 subjects had extremely high values. In this study, we had the opportunity to analyze the records of these infants in detail.

Table 1. Serum IgGoxLDL in mothers and in their children at birth and 3 months later.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mothers</th>
<th>Newborns</th>
<th>Mothers</th>
<th>Infants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast-fed (n = 6)</td>
<td>803 ± 375</td>
<td>657 ± 316</td>
<td>721 ± 297</td>
<td>84 ± 36</td>
</tr>
<tr>
<td>Formula-fed (n = 8)</td>
<td>1418 ± 1271</td>
<td>739 ± 605</td>
<td>1025 ± 818</td>
<td>4066 ± 2342</td>
</tr>
<tr>
<td>Total (n = 14)</td>
<td>1059 ± 874</td>
<td>691 ± 435</td>
<td>848 ± 562</td>
<td>1743 ± 2490</td>
</tr>
</tbody>
</table>

*Values expressed as mean ± SD.

Surprisingly, the infants with extremely high IgGoxLDL had not been breast-fed during the first 3 months of life, whereas the others had been (Table 1). The difference between the values in breast-fed and formula-fed infants was statistically significant ($P < 0.001$, Wilcoxon unpaired test).

The Spearman rank correlation between IgGoxLDL concentrations in the sera of mothers and their newborns was statistically significant ($r = 0.79$; $P < 0.001$); on the other hand, no significant correlation was found between values for 3-month-old infants and their mothers. The early correlation reflects transplacental transport of class G IgGoxLDL, whereas the infants were later able to produce their own antibodies. The production rate was individual and could be influenced by nutrition type. We also compared the number of DNA breaks in peripheral lymphocytes from 3-month-old infants by single-cell gel electrophoresis (comet assay).

This method in combination with endonuclease III (endoIII) treatment of cells is able to detect oxidized pyrimidines (2). When compared with breast-fed infants, formula-fed infants had a higher number of DNA strand breaks (0.39 ± 0.06 vs 0.14 ± 0.02 DNA strand breaks/10⁹ Da) and endoIII-sensitive sites representing predominantly oxidized pyrimidines (0.24 ± 0.10 vs 0.07 ± 0.04 endoIII sites/10⁹ Da; $P = 0.027$ and 0.007, respectively, Mann–Whitney). Because the nutrition of infants contributes for their future health (3), our preliminary data may be important.

There are several possible ways to interpret our findings. One possibility is that milk formulas contain higher concentrations of IgAoxLDL than breast milk; this explanation can be omitted because our method was sensitive only to IgGoxLDL. Another explanation for these results is that milk formula diminishes oxidative stress more than breast milk and consequently leads to increased free IgGoxLDL in the circulation of infants because these antibodies are unable to bind to oxidized LDL (4).

A completely different explanation is that formula feeding might cause some kind of gastrointestinal inflammatory reaction followed by early production of IgGoxLDL in these children. This may be likely if the milk formula is of nonhuman, e.g., bovine, origin, but we are also aware of the hypothesis that self-non-self recognition develops after the third month of life. On the other hand, our results show a significant increase of oxidative DNA damage in formula-fed infants compared with breast-fed infants. This increase may be a consequence of a higher oxidative load in formula-fed children, resulting from metabolic processes, or a consequence of higher antioxidative protection in breast-fed infants. The higher number of oxidized LDL particles, which are cytotoxic and able to induce apoptosis (5), may explain the enhanced oxidative DNA damage.

On the basis of our preliminary findings, we are not able to answer these questions properly, but we are convinced that additional studies should be initiated to get clearer recommendations regarding optimal nutrition for children during the first months of life.

References