Diagnostic markers of viral hepatitis B and C

C Trépo, F Zoulim, C Alonso, M-A Petit, C Pichoud, L Vitvitski

Abstract
Hepatitis B virus (HBV) serology has become extremely refined. As well as the recognised hepatitis B surface (HBs), hepatitis B core (HBc), and hepatitis B e (HBe) antigen-antibody systems, new markers have been introduced including pre-S1, pre-S2 for the envelope and the functional X protein. New automates have been introduced allowing flexibility in the different tests according to precise needs. The monitoring of pre-S1 antigen provides a relevant correlate of viral replication. The quantitative determination of HBV-DNA, pre-S1 Ag, and IgM anti-HBc seem most useful for the decision to use, and the monitoring of, antiviral treatment. Second generation ELISAs detect antibodies to three sets of hepatitis C virus (HCV) protein including the c22 core, and c35, and c100, which correspond to the non-structural regions (NS3 and NS4, respectively). Second generation ELISAs require confirmation by supplement assays, but their biggest limitation is the delayed appearance of anti-HCV after primary infection. In addition 10% of chronic infections with liver disease still remain seronegative despite circulating HCV RNA in serum or liver, or both. Much progress still has to be made before HCV serology can reach the level of sophistication of HBV.

(Gut 1993; supplement: S20–S25)

Hepatitis B

Overview
Although hepatitis B virus (HBV) infection has now been recognised for more than 25 years, its serology has evolved constantly and has become extremely refined with the significant advances of molecular biology and monoclonal antibody technology. In addition to the well known hepatitis B surface (HBs), core (HBc), and e (HBe) antigen-antibody (Ag/anti-) systems, new markers have been recognised, including pre-S1 and pre-S2 for the envelope and the functional X protein. Moreover, original tests have been made more quantitative and sensitive and therefore now have a greater clinical relevance. Finally, new automates have been introduced which allow flexibility in the hierarchy between different tests according to precise requirements.

The hitherto overlooked genetic heterogeneity of HBV, recently disclosed by molecular biology, has necessitated reconsideration of the classic serological distinctions between healthy carriers and progressive symptomatic or asymptomatic infection. Markers may be separated tentatively into two groups: those that are mainly diagnostic and those that allow assessment of prognosis and infectivity. HBsAg, anti-HBs, and anti-HBc should be used simultaneously for diagnosis. Moreover, in contrast with HIV or hepatitis C virus (HCV), it is often possible to confirm the specificity of one test within the HBV marker spectrum itself. For example, the specificity of HBsAg can be assessed by testing for anti-HBC or HBcAg/anti-HBc. This also applies to anti-HBs as a marker of immune elimination of HBV. In isolated anti-HBc, polymerase chain reaction (PCR) should be used to investigate the possibility of low grade ongoing HBV infection. Western blot profiles and peptide analysis also confirm the specificity and variability of the anti-HBc response.

New and Potentially Useful Markers of HBV Infection
Three additional coding regions leading to expression of protein during the life cycle of the virus have been studied intensively: pre-S1, pre-S2, and X. The polymerase gene product has received least attention. With suitable antibodies, the product of the polymerase gene can be shown in the liver of infected carrier patients. In a study of renal dialysis patients, anti-polymerase antibodies have been reported as the earliest marker of HBV infection. Both in human and experimental hepatitis virus infection in the woodchuck, anti-pol antibodies were constant in fulminant hepatitis and common in acute HBV infection as well as in chronic infection, where they correlated directly with the existence of ongoing HBV replication. Remarkably, the occurrence of anti-pol antibodies in chronic hepatitis B patients was about the same in those with HBeAg and those with anti-HBe. This is further evidence that ongoing viral replication in such patients may well persist in the presence of anti-HBe.

Several groups, including ours, have looked for hepatitis B x antigen (HBxAg) in the tissue and anti-HBx in the serum samples of patients with a variety of HBV-associated diseases. The results seem discrepant, but this is not surprising as different probes, either recombinant fusion protein or synthetic peptides, have been used by different authors. Results are in agreement regarding the transactivation function of the X protein. Expression of HBxAg in the liver has been confirmed universally. The detection of HBxAg on the plasma membrane of infected hepatocytes, together with a close correlation between HBxAg positivity and raised serum
Diagnostic markers of viral hepatitis B and C

alanine aminotransferase (ALT), implies that the HBxAg may be implicated in the pathogenesis of HBV infection. Another possibly relevant phenomenon is the activation of the expression of major histocompatibility antigens by HBxAg.

The detection of HBxAg in the serum has been reported by only two groups and is still awaiting confirmation. HBx antigenaemia seems to be restricted mainly to chronic HBV infection with positivity for HBV-DNA in serum and HBeAg in liver tissue.

Anti-HBs is detectable earlier and is found in both acute and chronic infection. Titres and prevalence correlate with the intensity of HBV replication. The correlation between anti-HBx and liver cancer has not been seen universally but this may be more a reflection of duration and intensity of replication than of carcinogenesis in itself. Seroconversion of HBxAg to anti-HBx positivity occurs in most patients before HBeAg to anti-HBe seroconversion. It is independent of the HBe antigen-antibody markers. The importance of the presence of HBxAg in the absence of other HBV markers requires further study.

Many more groups have evaluated pre-S antigen as a marker of viral replication. All studies seemed to find much higher values of pre-S antigen in HBV-DNA positive cases as compared with HBV-DNA negative cases, but the proportion of HBV-DNA cases reactive for pre-S varied according to the technique used.

Both the pre-S1 and pre-S2 domains have been shown to contain host receptor binding sites and to affect the attachment of HBV to hepatocyte receptors. It is believed that HBV may bind indirectly (by polymerised human serum albumin as an intermediate molecule) through the pre-S2 domain and directly to hepatocyte membrane through the pre-S1 domain.

Several studies using antibodies in competition experiments have identified the specific 21–47 region of the pre-S1 sequence (which involves 174 amino acids) as the receptor binding site. A double radioimmunoassay using specific monoclonal antibody recognising 21–47 has been developed on the envelope of HBV. Using this assay, the expression of pre-S1 antigen correlated well with levels of HBV replication in patients with chronic active hepatitis B.

To date, none of the new markers, anti-pol, HBxAg, anti-HBx, pre-S1, and pre-S2 antigens or antibodies have been made commercially available, although some prototypes have been developed.

Heterogeneity of HBV strains: importance of pre-core negative HBV mutants

In 1989, several groups, including ours, reported the identification of a pre-core defective HBV variant in patients with anti-HBe positive chronic active hepatitis. The pre-core mutation inhibits expression of HBeAg. Anti-HBe positivity may result from earlier infection with the wild type virus, or may represent a response to degraded core proteins. Even though the prevalence of such mutants has been studied in various regions of the world, showing a high proportion of mutations in Africa, Asia, and the Mediterranean countries, the biological and clinical consequences of the phenomenon remain unclear. Several reports focused on the severity of the infection with such mutated strains, including propensity to fulminant hepatitis and rapidly progressing chronic active hepatitis. We have recently confirmed the replication capacity of this HBV mutant and successfully shown by transfection the infectivity for chimpanzees. Although our results suggested decreased replication ability of pre-core negative mutated HBV variants, they confirmed the ability to infect both in vivo and in vitro. Remarkably, the infection with pre-core negative mutants is associated with cytoplasmic rather than nuclear expression of HBcAg. This provides indirect evidence of the existence of HBeAg variant strains. The existence of several populations of wild type and mutated HBV strains in patients, as well as fluctuation of such populations during the natural course of the disease or following treatment, has already been reported. Whether some HBeAg negative variants are true 'escape mutants' and a result of immune selection, or whether they derive from direct exogenous infections needs to be studied further.

It is important to emphasise that interferon acts, in part, by immunomodulation and thus may contribute to the selection of such an escape mutant. The response of chronic active hepatitis to interferon has been shown to be related indirectly to the proportion of wild type and HBeAg negative mutants. Therefore, the detection of these mutants is now necessary for the successful management of HBV infection. It is important to remember that because of a lower HBV replication capacity, HBeAg negative infection is often associated with progressive liver disease with evidence of HBV replication in the liver, even though serum HBV-DNA tests are negative. Such infections may represent 50% or more of the cases studied at one time. The management of anti-HBe positive liver disease is extremely difficult with the markers currently available as HBeAg is dismissed and HBV-DNA is present in less than half of the patients at a titre detectable by conventional hybridisation techniques. We have shown that, in such cases, study of the pre-S1/HBsAg ratio is invaluable. Indeed, the pre-core negative mutation does not affect the viral envelope. Table I lists the investigations required for the management of chronic liver disease associated with HBeAg negative mutants.

### Table I. Specific investigations for the management of chronic liver disease associated with HBeAg negative mutants

| 1 | HBeAg disqualified |
| 2 | HBV-DNA on PCR (undetectable by conventional tests in >95%) |
| 3 | Anti-HBc IgM/ultrasensitive (correlates with disease activity) |
| 4 | High Pre-S1/HBsAg ratio (correlates with replication) |
IMPORTANCE OF SERUM IGM ANTI-HBC IN CHRONIC HEPATITIS VIRUS B INFECTION

Although the classic pattern of antibody response to acute viral infection is characterised by an early IgM response and the appearance of secondary IgG-specific antibody, it is also known that IgM antibodies to viral antigen often persist in chronic viral infection with ongoing viral replication. It is still not clear whether fluctuation of low values of IgM anti-HBc antibody can be correlated with the intensity of HBV replication or severity of histological liver disease. It should be emphasised that ultrasensitive tests are required to assay monomeric IgM anti-HBc titres in chronic infection. It is notable that, in one study, although no correlation could be found between IgM anti-HBc and serum markers of active viral replication or HBsAg titres in 49 patients with chronic HBV infection, a significant association was seen between intrahepatic expression of cytoplasmic HBeAg and active liver histology. By including the samples only slightly reactive for IgM anti-HBc, a significant difference was found between cases of chronic hepatitis and asymptomatic carriers. That is, patients with chronic hepatitis were more likely to be IgM anti-HBc positive than asymptomatic carriers of HBV.

Given the many tests available, it is critical to select only the minimum necessary to allow assessment of the clinical situation, including HBV variants. The need for accurate processing of a large number of samples prompted us to evaluate fully automated enzyme immunoassays (IMX Abbott, Chicago, IL, USA) for the assessment of both HBsAg and IgM anti-HBc. HBV-DNA titre was also assessed quantitatively using a solution hybridisation test.

To assess the clinical relevance of these assays, they were compared with anti-HBs, pre-S1, and pre-S2 antigen detection. Three groups of patients were studied, corresponding to the main clinical situations in chronic infection: (a) healthy asymptomatic chronic HBsAg carriers with normal liver function tests; (b) chronic hepatitis B patients with active replication and progressive liver disease, with two subgroups: (i) patients with HBsAg and high HBV-DNA, and (ii) those with anti-HBe and low HBV-DNA values in serum.

Serum HBsAg values detected by IMX were higher in HBsAg positive cases than in anti-HBe positive carriers. In all subgroups, serum HBsAg values correlated with those of serum HBV-DNA. The detection of low levels of IgM anti-HBc by IMX was associated with the presence of liver disease but not with the level of viral replication. The prevalence of anti-HBx was slightly, although not significantly, higher in patients with high replication (>100 pg/ml). As mentioned before, monitoring of anti-HBe positive chronic active hepatitis requires the use of additional markers.

**Chronic HBV infections**

Chronic HBV infections are those which last more than six months and are classified into wild type or mutated genotype. One of the

<table>
<thead>
<tr>
<th>Pre-S1 Ag</th>
<th>PCR/ethidium bromide staining</th>
<th>PCR/southern blot hybridisation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Positive (n=24)</td>
<td>24 (100%)</td>
<td>0</td>
</tr>
<tr>
<td>Negative (n=23)</td>
<td>5 (22%)</td>
<td>18 (78%)</td>
</tr>
</tbody>
</table>

**Primary infection**

Once exposure to HBV has been shown and infection proved, it is necessary to distinguish a recent infection from an exacerbation occurring in a chronic carrier. This can be done by IgM anti-HBc titre. The increased sensitivity of this test, which allowed the introduction of a cut off value to distinguish accurately between chronic and acute infections, has redefined its significance. To date, we have assumed that all primary infection was of the wild type but, in future, we should consider whether pre-core negative mutant or mixed infections are present.

**Prolonged hepatitis B**

Prolonged hepatitis B is defined as the persistence of abnormal serum ALT activities for more than six weeks but less than six months. The condition has to be monitored carefully and treated adequately. Evidence indicates that, whenever HBsAg and HBV-DNA persist, the chances of spontaneous recovery are almost non-existent. In contrast, interferon treatment may be highly successful at this stage, as suggested by an interim analysis of an ongoing controlled study.
most common mutations is the HBeAg negative variant, which may be associated with different types of mutation in the pre-core region, all of which inhibit HBeAg expression and release. In this case, the HBeAg/anti-HBe system is invalidated as a prognostic and replication marker. An updated definition of the healthy HBV carrier state is therefore crucial, with careful distinction between healthy HBsAg carriers and those with asymptomatic or symptomatic liver disease, assessed according to the new assays (Tables III and IV). Obviously, the liver function tests must be normal, anti-HBe should be positive, and HBV-DNA should be negative at least by conventional methods. IgM anti-HBc and pre-S1/HBsAg ratio should also be negative by ultrasensitive assays. Chronic hepatitis may differ from the above profile in that ALT may be raised at anytime. Inflammation can be tested for by liver biopsy, a procedure that is unnecessary in patients with the previous profile. Pre-S1 antigen and IgM anti-HBc will be positive, while positivity will vary for HBeAg, in cases of wild type infection, or anti-HBe, and in cases of pre-core negative variant infection.

**The hepatitis B immunity profile**

The hepatitis B immunity profile after natural infection requires the simultaneous positivity of both anti-HBs and anti-HBc and, possibly, of anti-pre-S. In cases of vaccination, only anti-HBs and, eventually, anti-pre-S (depending on the type of vaccine) are found.

**Reactivation**

This term should be restricted mostly to a specific situation in which patients with wild type infection have transiently cleared HBeAg and HBV-DNA and, in a limited number of cases, pre-S Ag antigen; second phase replication then resumes with the reappearance of HBeAg and HBsAg, or both, as well as HBV-DNA. Reactivation can even be seen after seroconversion from HBsAg to anti-HBs, but only in cases of immunodepression after HIV infection or immunosuppressive treatment. In cases of infection with mutated HBe(-) variant, many flares are seen and are part of the natural history of the infection. The term ‘reactivation’ should not be used loosely for every exacerbation in cases such as these.

### CONCLUSIONS

We have carried out extensive analysis of the existing HBV markers, which have been improved by quantitative determination methods, to assess accurately HBV replication and to monitor its fluctuation in chronic HBV infection with both wild type or HBeAg negative variants before, during, and after antiviral treatment. The results indicate that, in addition to HBSAg, the most critical variants are pre-S1 antigen and HBV-DNA. In general, the quantitative determination of HBV-DNA, pre-S1 antigen, and IgM anti-HBc seems most useful for the decision to treat, and the monitoring of, antiviral treatment. This is especially true for anti-HBe positive carriers with liver disease, as HBV-DNA values are often too low to be readily detectable.

Persistence of pre-S1 antigen and IgM anti-HBc, or both confirm ongoing HBV replication, as shown by PCR, and rule out the true healthy carrier state. Therefore, there is a much smaller proportion of cases than previously thought that fall into this category. Strict criteria should be maintained, however, to identify a much larger proportion of patients with minimal liver disease who should be monitored to recognise early reactivation or emergence of mutants. Such cases could be treated more effectively if treatment was started earlier.

### Hepatitis C

The real breakthrough in terms of sensitivity was achieved when it became possible to add a second adjacent non-structural protein (NS3 or c33) and a structural protein from the viral capsid or core (c22) to the c100-3 NS4 protein. This set of three proteins was selected carefully after extensive testing, and two major diagnostic companies (Ortho and Abbott) are presently distributing second generation ELISAs based on the association between the three. Other manufacturers which use distinct recombinant proteins (Pasteur Diagnostic and Wellcome) or combined synthetic peptides (UBI Organon) are accessing the market, but they have yet to offer strong competition with the market leaders. Results from second generation assays are only beginning to be reported in studies and most references still document first generation assays. The principle of anti-hepatitis C virus (HCV) ELISA, first and second generation, is based on a ‘sandwich’ antiglobulin assay in which recombinant proteins or synthetic peptides fixed on a solid phase bind the antibodies present in the serum. Those antibodies are shown by anti-human IgG. As for HIV tests, this type of assay is associated with specificity

### TABLE II

<table>
<thead>
<tr>
<th>Markers</th>
<th>Anti-HBe positive, asymptomatic (n=23)</th>
<th>Anti-HBe positive, chronic hepatitis (n=25)</th>
<th>HBeAg positive, chronic hepatitis (n=40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBsAg</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Pre-S1Ag*</td>
<td>61</td>
<td>100</td>
<td>98</td>
</tr>
<tr>
<td>Pre-S2Ag</td>
<td>100</td>
<td>100</td>
<td>98</td>
</tr>
<tr>
<td>IgM anti-HBc</td>
<td>17</td>
<td>24</td>
<td>18</td>
</tr>
<tr>
<td>Anti-HBx</td>
<td>45</td>
<td>29</td>
<td>47</td>
</tr>
<tr>
<td>HBV-DNA*</td>
<td>9</td>
<td>44</td>
<td>80</td>
</tr>
</tbody>
</table>

*p=0.0001.
problems; any antibody to protein which may be fixed together with HCV specific protein will also react and will need to be assessed further by supplementary testing. Therefore, HCV screening must include initial assays with maximum sensitivity followed by confirmatory assays providing the necessary specificity. Sensitivity and specificity in HCV infection are still limited, as will become apparent.

The increased proficiency associated with the use of second generation assays is important, although variable, and depends on the clinical setting. For example, in the screening of blood donors in France, the number of positive samples in low prevalence areas almost doubled when second generation tests were used. In high risk groups, such as drug addicts, an increase in true positives from 5 to 40% was seen with second generation assays. Among patients with chronic non-A, non-B (NANB) hepatitis, the increase of 5 to 15% brought the final anti-HCV reactivity rate to over 85%. Among patients with acute sporadic NANB hepatitis, the improved rate of detection was about 20%. In prospective studies after transfusion, anti-HCV was detected 30–90 days earlier with second generation assays, and it is now clear that all cases seroconverted within five months after transfusion. Despite these benefits, between 20 and 40% of cases of presumed acute hepatitis C remain anti-HCV negative on tests carried out at the start of symptoms or at peak ALT activity. The higher reactivity rate associated with sporadic acute hepatitis may partly be a result of misinterpretation of established chronic infection. In contrast with the delayed appearance of anti-HCV, detection of HCV-RNA on PCR is an early event, occurring as soon as five to 10 days after transfusion. In addition, 10% of patients with chronic hepatitis C may be seronegative for anti-HCV; in such cases, only HCV-RNA can be shown by PCR. The prevalence of anti-HCV negativity is increased in cases of immunodeficiency linked to infection or transplantation. These findings are similar to those reported in studies of maternal transmission of HCV from mother to child, in which HCV viraemia was seen in the absence of any antibody. Moreover, a recent study comparing all commercially available HCV screening tests (ELISA second generation) found that 32% of blood donors with raised ALT greater than 100 IU/l had detectable HCV-RNA in the absence of any detectable antibody.23

SPECIFICITY

Many papers have reported a very high frequency of false positives associated with anti-HCV testing.24–31 Despite the improved sensitivity of the second generation tests, they remain subject to the same specificity problems as those of the first generation, as their configurations are identical. In prospective epidemiological surveys, only 50% of anti-HCV positive blood donors were actually found to be infectious. Only 50–75% of serum samples anti-HCV positive by second generation tests can be confirmed as positive by second generation recombinant immunoblot assays (RIBA-2), with about one third truly confirmed and 25% with indeterminate (single-band) patterns.32

Another common cause of false positives is hypergammaglobulinaemia occurring, for example, in serum samples from patients of African origin and those with rheumatoid factors, as well as from patients with myeloma, hepatocellular carcinoma or chronic liver disease, but especially from those with autoimmune chronic active hepatitis.24 In this last group, superoxide dismutase antibodies have also been found. As the HCV proteins are cloned in yeast with superoxide dismutase antibodies as a fusion protein, the false positive cross reaction is conceivable.30 In addition, false positives have been reported following repeated freeze thaw cycles or when stored for long periods at unstable temperatures. Other questions need to be answered, such as the role of anti-idiotypes, immune complexes, and other, as yet unidentified, factors which modify the physicochemical properties of serum proteins and may increase their interaction with gammaglobulins and with the solid phase of the assay, or both.

Thus, because of their limited specificity, each anti-HCV assay must be followed by a complementary ‘confirmation’ test. Several such complementary assays are now available and are described below. To date, the immunoblot type tests have been used most commonly, in which the structural protein c22 and non-structural proteins c33 and c100 are coated as separate bands on nitrocellulose strips (RIBA, Ortho, Raritan, New Jersey, USA) or disks (MATRIX, Abbott, Chicago, IL, USA). These tests have excellent specificity but lack sensitivity. Among blood donors, they yield too many indeterminate results; 30% detect only one of the proteins (c22 or c33 antibodies) and yet two proteins are required for a true HCV infection. The ‘two beads’ assay (Abbott) consists of two separate beads coated with c22 (structural) and c33+c100 (non-structural) proteins. This assay has good sensitivity and specificity, and scores positive if the sample is reactive with only one bead.

The conflicting interpretations of serological data require clarification. Some suggest that a blood donor positive for only one protein may well be truly infected with HCV, whereas others suspect a false reaction as a likely cause of this commonly seen pattern. Indeed, most blood donors with only one protein are not infectious, but this does not mean that they have not been exposed to HCV. They may retain a residual anti-c22 reactivity resulting from the sequential loss of c33 and, later, c100 antibodies, as the c22 antibody seems to be the most persistent. In liver disease patients, however, most cases of anti-c22 or anti-c33 single reactivity are hepatitis C viraemic, as indicated by PCR. Thus, it seems important to assay RIBA-2 indeterminate samples by PCR to confirm HCV infection.
PCR testing should also be performed in autoimmune groups, as chronic active autoimmune hepatitis is commonly associated with false positive reactions. Two types of autoimmunity hepatitis must be distinguished: type 1, associated with smooth muscle antibodies of the anti actin type, and in which anti HCV is usually non-specific, non-neutralising, and not confirmed by immunoblot; type 2, characterised by the presence of anti liver kidney microsomal 1 antibodies, with confirmation of anti HCV by immunoblot in more than 75% of cases. As treatment of HCV infection with interferon exacerbates the autoimmune reactions, the search for HCV RNA by PCR in the serum, lymphocytes, or liver seems imperative in autoimmune diseases associated with anti HCV positivity. The search for HCV RNA also seems necessary in acute or chronic anti HCV seronegative hepatitis. Immunostaining techniques capable of showing HCV proteins in the liver should be developed further.

It should be emphasised that the 'confirmatory tests' cannot be expected to reflect the actual virainema, nor can they be used after antiviral treatments, as they predict neither the probability of response nor relapse. Therefore, it is necessary to develop new tests with additional proteins distinct from those used in the ELISA, to meet the prerequisites of a true confirmatory assay.

Conclusions
HCV serology is evolving rapidly, but we still need to be aware of both false positive and false negative anti HCV results. All positive screening tests should be confirmed by supplemental assays such as RIBA-2, MATRIX, or double bead type assays. Indeterminate results should be confirmed by investigation of HCV RNA using PCR, particularly in patients with anti HCV positive autoimmune hepatitis. Immunostaining of the liver and anti HCV IgM are showing great promise, but we still need serological markers to distinguish between immune patients who have cleared HCV, so-called 'healthy carriers', and infectious patients with replicating virus and progressive disease. Thus, HCV serology requires considerable refinement before reaching the level of sophistication achieved for HBV.

Diagnostic markers of viral hepatitis B and C.

C Trépo, F Zoulim, C Alonso, M A Petit, C Pichoud and L Vitvitski

Gut 1993 34: S20-S25
doi: 10.1136/gut.34.2_Suppl.S20

Updated information and services can be found at:
http://gut.bmj.com/content/34/2_Suppl/S20

Email alerting service

Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/