

prevalence of smoking among the controls in the study by Tanis et al. is representative of the population as a whole, then nearly three fourths of the cases of myocardial infarction (74.7 percent) can be attributed to smoking among women in this age group in the Netherlands. Similarly, Dunn et al. estimated that 73 percent of myocardial infarctions among young women in the United Kingdom were attributable to smoking.¹⁰

In summary, increasing evidence suggests that third-generation oral contraceptives are indeed safer than previous formulations in terms of the risk of cardiovascular disease. Any increase in the risk of myocardial infarction among current users is small, and past users of oral contraceptives (regardless of the generation) have no lingering risk from that exposure.¹²

With appropriate treatment for hypertension and elevated lipid levels, along with moderate changes in lifestyle, the majority of cases of coronary disease — still the number-one cause of death in the United States — could be prevented.¹³ Smoking, therefore, remains of preeminent importance. Cigarettes are the only product sold on the free market that, when used as directed, can kill people. Research funding and clinical effort must now place greater priority on improving ways to aid smoking cessation and on reducing the number of young people who take up smoking.

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HLA MATCHING FOR BONE MARROW TRANSPLANTATION — HOW MUCH IS ENOUGH?

DESPITE the very large number of volunteers willing to donate bone marrow for unrelated recipients (currently numbering over 7 million worldwide), many candidate recipients still cannot obtain marrow because no HLA-matched volunteer can be found. The major histocompatibility complex (MHC), a group of closely linked genes on chromosome 6, encodes the class I and class II HLA molecules, which, in concert with T-cell receptors, make possible the immune recognition of foreign antigens. HLA molecules are also alloantigens that can trigger immune recognition and graft rejection in unmatched recipients.¹

HLA antigens in allografts, in allogeneic leukocytes in transfusions, and in fetal cells that enter the maternal circulation can induce the formation of alloantibodies against discrete HLA antigens. Such antibodies formed the basis for the serologic definition of numerous class I antigens and the assignment of their genetic determinants to particular MHC gene loci.²

However, serologic techniques and reagents cannot reveal all currently known HLA molecular variants. Whole families of alleles whose HLA products share serologic markers have been found to encode distinct molecular variants. They can be distinguished by high-resolution techniques, including direct sequencing of the corresponding alleles and other sequence-based methods.³ HLA variants discernible only with molecular tools are sometimes designated "alleles," to distinguish them from "antigens" — serologically recognizable variants that can elicit alloantibodies. (I will use the term "allele" in quotation marks to indicate this somewhat arbitrary sense. In contrast, the term allele, without quotation marks, will be used to convey the common genetic meaning of an alternative form of the gene at a given locus.) Despite the absence of serologic signatures, class I "allele" mismatches can induce the generation of killer T cells that cause immune rejection of mismatched grafts.⁴ Molecular HLA identity between unrelated

donors and recipients of bone marrow, approaching that which exists between HLA-identical siblings, seems to provide the best chance for avoiding graft rejection and other serious complications of bone marrow transplantation.^{5,6}

For these reasons, there is an enormous effort to define and characterize new HLA alleles. As a consequence, the number of alleles has grown and continues to grow at a sustained pace, making the polymorphism of the linked genes of the HLA complex truly astounding. There are three class I gene loci, HLA-A, B, and C, and thus far more than 220 HLA-A, 460 HLA-B, and 110 HLA-C alleles have been described.⁷ Theoretically, this number of alleles can combine to produce over 11 million different HLA-A, B, C haplotypes and billions of possible diploid combinations. Polymorphism is also a feature of class II genes, and class II matching is required in the selection of bone marrow donors: DRB1 has more than 360 alleles, DQA1 has 22, DQB1 48, DPA1 20, and DPB1 96.⁷ Even though some of the DNA sequences of these alleles do not encode different amino acid sequences, the chance of finding an unrelated donor who matches a recipient for all class I and class II proteins is extremely small, except for patients whose HLA haplotypes are very frequent in the population. Most patients who need a bone marrow transplant from an unrelated donor thus have no real chance of finding a completely matched donor and must receive partly mismatched grafts.

The hope that some mismatches may be so slight as to be “permissible” — that is, less clinically troublesome than others — finds support in a retrospective analysis of the influence of HLA “allele” incompatibility on the rejection of marrow grafts from unrelated donors. The analysis by Petersdorf et al., reported in this issue of the *Journal*,⁸ indicates that a single mismatched “allele” does not cause a measurable increase in the probability of graft failure, whereas single mismatched antigens or combinations of two or more mismatches for “alleles” or antigens do. Through comparison with the crystallographic model of the class I molecule,⁹ the location of the variant amino acid sequences in the mismatched “alleles” and antigens was deduced to be in the peptide-binding region of the class I molecule, in the complementarity-determining region for the T-cell receptor, or to a lesser degree elsewhere in the molecule. Petersdorf et al. suggest that the type and location of the mismatched amino acid residues in class I molecules are important in determining whether single HLA class I mismatches may be clinically permissible.⁸ Another consideration is that single antigen mismatches usually entail more amino acid substitutions than single “allele” mismatches. Thus, there may be qualitative as well as quantitative thresholds of class I molecular incompatibility that must be exceeded for rejection to occur.

The number of molecular mismatches may also be associated with different degrees of risk of graft rejection. In one study, recipients mismatched for a single antigen and homozygous at that locus had a greater probability of graft failure than heterozygous recipients.¹⁰ Petersdorf et al. suggest that homozygosity reduces the number of the recipient’s class I molecular targets against which the donor’s immune cells can react. Fewer class I targets in the recipient would then tip the balance toward rejection and away from graft-versus-host disease as the immune cells of the donor and the recipient react against one another.⁸

These remarkable observations suggest new strategies for the selection of bone marrow donors that could improve the odds of engraftment by categorizing single HLA class I mismatches according to their implications for the risk of rejection. First, if the recipient is homozygous for an HLA allele, donors with a single HLA mismatch who are also homozygous for the same allele are preferable. Second, when the single mismatch involves only an “allele” (e.g., patient type, A*0202, donor type, A*0203), the mismatch predicts no increase in the odds of rejection, and this donor could be accepted as if fully matched. By contrast, a single antigen mismatch (e.g., patient type, A*0202, donor type, A*0302) does increase the risk of rejection, and a donor with no antigen mismatches should be sought.

The observations of Petersdorf et al. deserve further study to clarify any requirements for pretransplantation conditioning or prophylaxis against graft-versus-host disease that may be necessary for long-term engraftment of “allele”-mismatched grafts. They also call for in-depth analyses of postengraftment outcomes for recipients of transplants with “allele” mismatches, including the development of acute and chronic graft-versus-host disease, leukemic relapse, and survival.

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RATIONAL USE OF ANALGESIC AND ANTIINFLAMMATORY DRUGS

“TAKE two aspirin and call me in the morning?” Clinical decision making is no longer so straightforward. Millions of patients take analgesic and anti-inflammatory medications regularly. The reasons are simple: these drugs are effective in relieving pain and inflammation,¹ and aspirin provides protection from cardiovascular thrombosis.^{2,3} Osteoarthritis and other painful chronic conditions occur in a population of patients who often have coexisting medical conditions, including conditions associated with an increased risk of cardiovascular disease. Thus, aspirin is frequently prescribed for patients taking other analgesic and anti-inflammatory agents. Since the mechanism of action of all these drugs is the inhibition of the cyclooxygenase (COX) enzymes, their adverse-event and efficacy profiles may change when they are used in combination.

The discovery of cyclooxygenase-2 (COX-2) represented an enormous conceptual advance in prostaglandin biology and provided new therapeutic options.^{1,4,5} Acetaminophen, traditional nonsteroidal anti-inflammatory drugs (NSAIDs), and specific COX-2 inhibitors (coxibs) are all currently recommended for the treatment of osteoarthritis.⁵ When prescribing these drugs, physicians must consider their efficacy, but their toxicity and cost have appropriately become major considerations as well.^{1,4} Although the relative effect of these drugs on gastrointestinal ulcers and bleeding has attracted the most attention,⁶⁻⁸ cardiovascular and renal complications have recently assumed importance in the evaluation of their side effects, since the COX enzymes have prominent biologic roles in the vasculature and the kidneys.^{9,10} In this issue of the *Journal*, Catella-Lawson et al.¹¹ address the potential for a competitive interaction between aspirin and other analgesic and anti-inflammatory agents, and Foreed et al.¹² examine the role of acetaminophen and aspirin in the progression of renal disease to chronic renal failure.

There are important distinctions among the mechanisms of action of aspirin, NSAIDs, coxibs, and acetaminophen. Aspirin remains unique among NSAIDs as an irreversible inhibitor of the activity of the COX

enzymes. Low-dose aspirin acts as a selective inhibitor of platelet cyclooxygenase-1 (COX-1) activity by virtue of the fact that platelets, in contrast to nucleated cells, cannot recover COX activity.^{4,11} Nonaspirin NSAIDs inhibit the activity of both COX-1 and COX-2 by reversibly blocking the access of arachidonic acid to the active site at the apex of a hydrophobic channel within these enzymes. The pharmacodynamic properties of the different NSAIDs with respect to the COX enzymes vary with their chemical structures.⁴ Coxibs achieve specificity by virtue of a structure that is accommodated more efficiently by COX-2, which has a larger hydrophobic channel.¹ Acetaminophen is a weak, nonselective inhibitor of both COX enzymes. The precise mechanism of action of acetaminophen remains elusive, but acetaminophen does not appear to block the hydrophobic channel.¹³ It was recently proposed that acetaminophen acts to reduce the active, oxidized form of the COX enzymes, which would make it more potent at sites, such as the brain and spinal cord, that have low peroxide concentrations.¹³

Catella-Lawson et al.¹¹ proposed the interesting and important hypothesis that by occupying the hydrophobic channel of platelet COX-1, NSAIDs could interfere with the antiplatelet effect of aspirin. The investigators demonstrated that prolonged dosing with ibuprofen blocked the inhibitory effect of low-dose aspirin on the release of thromboxane by platelets and platelet aggregation. This competitive interaction could not, however, be generalized to all NSAIDs, since experiments in which delayed-release diclofenac, rather than ibuprofen, was used failed to yield similar results. Acetaminophen and the specific COX-2 inhibitor rofecoxib, which do not block the COX-1 hydrophobic channel, did not interfere with the effect of low-dose aspirin on platelet function.

The hypothesis that some NSAIDs are competitive inhibitors of aspirin with respect to platelet function requires further clinical evaluation. The platelet-aggregation studies reported by Catella-Lawson et al. were performed *ex vivo* and tested platelet function in isolation. Other factors may well contribute to the overall vascular effects of these drugs. For example, in an experimental model of thrombosis in animals, inhibition of COX-2 significantly decreases the coronary vasodilator response to infused arachidonic acid, irrespective of whether COX-1 activity is inhibited by aspirin. In the same model, inhibition of COX-2 prevents the antithrombotic effect of aspirin.¹⁴ Whether concurrent treatment with analgesic and anti-inflammatory agents blunts the cardiovascular protective effects of aspirin has not been determined in human studies. Thus, *in vivo* and clinical studies assessing the combination of low-dose aspirin with NSAIDs, coxibs, or acetaminophen will be required to determine the cardiovascular implications of the interactions among these drugs.