HLA-B7 by 50% (IC_{50}) was 34 nM, whereas the IC_{50} for the SMCX peptide was 140 nM (Fig. 3B). Thus, the significant difference in the ability of the SMCY and SMCX peptides to sensitize targets for T cell recognition is almost entirely due to the fine specificity of the T cell receptor, rather than the differences in MHC binding affinities. The SMCX peptide was also present in naturally processed peptide extracts of HLA-B7, although its abundance was only 25% of the SMCY peptide abundance (*17*). Therefore the peptide epitope representing the HLA-B7–restricted H-Y antigen is derived from the protein encoded by *SMCY*.

The location of the *SMCY* gene and the control of its expression fit well with those expected of the H-Y antigen based on previous work. Expression of SMCY has been detected in all male tissues tested, as has H-Y (*4*, *7*, *19*). Deletion mapping in humans has placed the *HY* locus in a portion of interval 6 on the long arm of the human Y chromosome (*22*), and *SMCY* maps to this same interval (*20*). Our work also establishes that the H-Y structural gene is encoded on the Y chromosome, rather than being an autosomal gene controlled by Y. The SMCY and SMCX proteins are 85% identical, and the *SMCX* gene is expressed from both the active and the inactive X chromosomes in both mice and humans (*19*, *23*). Therefore, self-tolerance to SMCX will limit the number of SMCY peptides that could give rise to H-Y epitopes in association with different MHC molecules. On the other hand, SMCY contains almost 1500 residues, and the over 200 amino acid sequence differences between it and SMCX are scattered relatively uniformly throughout its length. Thus, a large number of distinct SMCY-specific peptides could be generated as H-Y epitopes. Whether the H-Y epitope peptides presented by other MHC molecules are also from *SMCY* is unknown, because genetic mapping of the mouse Y chromosome has suggested between two and five distinct loci encoding H-Y antigens (*24*). However, a murine H-Y epitope restricted by $H\text{-}2K^k$ has also been shown to be derived from the murine Smcy protein (*25*). The demonstration that two H-Y epitopes from either mouse or human are derived from the same protein makes SMCY the prime target in searching other H-Y epitopes.

The identification of the protein that gives rise to an H-Y antigen culminates 40 years of uncertainty regarding its origin and many attempts to identify it. The 77% DNA sequence identity between SMCY and SMCX provides a likely explanation for past failures to identify H-Y–encoding genes by subtractive hybridization. Both proteins share significant sequence homology to retinoblastoma binding protein 2, which has been suggested to be a transcription factor (*26*). If SMCY functions as such, its presumed intracellular location would be inconsistent with detection by male-specific antibodies that have been shown to recognize cell surface structures (*27*). Although the function of SMCY, as well as the homologous SMCX, remains unclear, this and other H-Y specific peptides are candidates for immunomodulatory approaches in bone marrow transplantation, genetic probes to be used for prenatal diagnosis in sex-linked congenital abnormalities, and investigating minimal residual disease and chimerism.

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29. Supported by U.S. Public Health Service grants AI20963 (to V.H.E.) and AI33993 (to D.F.H.), the J. A. Cohen Institute for Radiopathology and Radiation Protection (E.G.), the Niels Stensen Foundation (E.G.), and the Dutch Organization for Scientific Research (NWO 901-09-201 to J.M.M.d.H.).

14 July 1995; accepted 21 July 1995

TECHNICAL COMMENTS

Neutrophilia in Mice That Lack the Murine IL-8 Receptor Homolog

G. Cacalano *et al.* describe neutrophil and B cell expansion in mice lacking the murine interleukin-8 receptor homolog (mIL-8Rh) (*1*). Neutrophils from these mice did not migrate toward ligands of the mIL-8Rh, and many fewer neutrophils arrived at sites of inflammation. These results could be expected, but the profound increase in the neutrophil and B cell populations was unexpected. Cacalano *et al.* offer several possible explanations for this result, but strong evidence to support any one is lacking.

We would like to offer an alternative explanation, namely, that the neutrophil and B cell expansion are compensatory changes for poor resistance to normal flora and pathogen exposure. We base this argument on functional, histological, and clinical similarities between these mice and patients with leukocyte adhesion deficiency (LAD). Humans, dogs, and cattle can have LAD, and all afflicted individuals suffer a defect in the CD18 gene and lack expression of β_2-integrin adhesion molecules on their neutrophils (*2*). Consequently, neutrophils are unable to adhere to and cross the endothelium, so they cannot reach sites of infection. Individuals with LAD may appear generally normal, especially when bacterial exposure is minimized, but they often suffer chronic, subclinical infections. Classical signs of LAD include gingival infection with abnormal dentition, and among cattle, growth retardation (*3*, *4*). Increased size of lymphoid organs and profound persistent neutrophilia with extensive granulopoietic activity outside of the bone marrow are hallmarks of this disease (*5*). Similarly, persistent neutrophilia

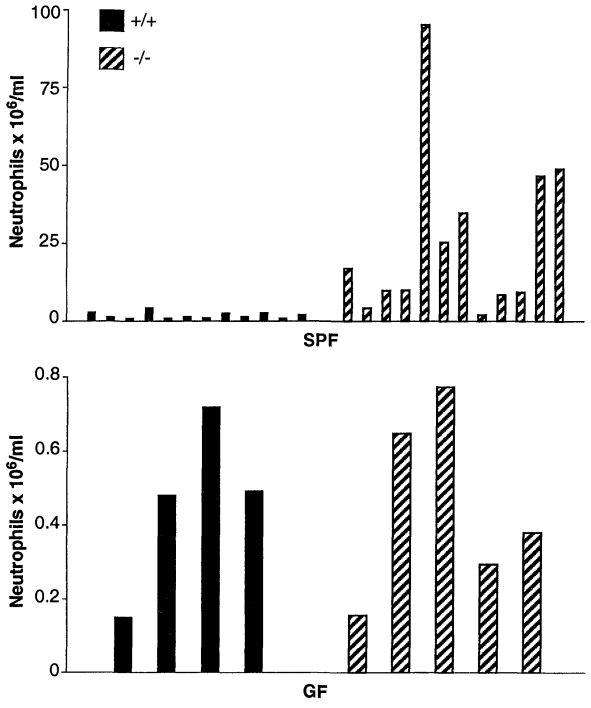

Fig. 1. Peripheral blood neutrophils from IL8Rh(+/+) and (−/−) mice derived under SPF and GF conditions. Blood was collected by retro-orbital venous puncture and was analyzed in a Serono-Baker Diagnostics System 9000 Diff Model Hematology Analyzer. Blood smears were stained with hematoxylin and eosin for differential cell counts performed microscopically. The total number of neutrophils was determined by multiplying the percentage of neutrophils by total white cell counts. Bars represent individual mice.

and periodontal disease are also observed with LAD type II where β_2-integrin expression is normal, but neutrophils have altered L-selectin expression and reduced efficiency to egress into tissues (*6*). Our experience with LAD cattle suggests that cytokine and chemoattractant production by tissues encountering normal flora or pathogens is not effectively down-regulated, as the eliciting agents are not removed by normal inflammatory processes. Not down-regulating inflammatory cytokine production (for example, granulocyte-macrophage colony-stimulatory factor) in infected tissues could be expected to result in the observed histopathological changes in the host, such as a progressive neutrophilia. In support of this hypothesis, the neutrophilia is much lower in LAD animals raised under germ-free conditions (*3*) than they are in animals raised conventionally.

The work of Cacalano *et al.* shows that mice lacking the mIL-8Rh, like LAD patients, suffer a marked impairment in neutrophil recruitment, and the hematological and histological changes in these mice are nearly identical to those associated with LAD. Furthermore, some of these mice suffer growth retardation and abnormal dentition (*7*).

Together, these data suggest that a severe impairment to neutrophil recruitment, whether through absence of adhesion molecules or chemoattractant receptors, reduces host defense to normal bacterial exposure and leads to compensatory changes in the immune system. This conclusion leads to the more significant implication that ligands of the mIL-8Rh must be essential for the surveillance function of neutrophils, at least in mice. Apparently, none of the other neutrophil chemoattractants—for example, leukotriene B4, complement fragments, or bacterial products—enable adequate neutrophil recruitment for normal host defense in the absence of the mIL-8Rh.

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23 February 1995; accepted 2 June 1995

Response: Shuster *et al.* point out some similarities in the phenotype of leukocyte adhesion deficient (LAD) animals and the IL-8 receptor–deleted mice [IL-8Rh(−/−)] that we have generated. There are some differences in the two animal models. In the LAD animals, adhesion and extravisation are blocked, whereas in the IL-8Rh(−/−) mice, only the chemokine-stimulated migration is affected. The IL-8Rh(−/−) mice do not show the extreme susceptibility to infections that occurs in LAD animals. In both cases, the net result is a dramatic increase in neutrophil production. Thus, one can hypothesize that blocking neutrophil function causes a stimulation of granulopoiesis in an apparent attempt to replenish the "lost neutrophil activity." In the discussion portion of our report, we raised the possibility of compensatory mechanisms being responsible for the elevated neutrophils and plasmacytosis. In order to test directly whether an impaired pathogenic response is involved in the increased neutrophil production, we compared IL-8Rh(−/−) mice bred in our conventional specific pathogen–free (SPF) environment to those rederived in a germ-free (GF) condition. Our initial results indicate that in GF conditions, the blood level neutrophils are not elevated (Fig. 1). This result shows that environmental pathogens are required for the neutrophilia observed in the IL-8Rh(−/−) mice. This may indicate that the inability to properly survey tissues and eliminate external pathogens results in the release of cytokines that, in turn, stimulate neutrophil production.

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24 March 1995; revised 19 July 1995; accepted 31 July 1995