PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **179**, 492–496 (1985)

Queuosine Metabolism: Possible Relation to B-Cell Activation by C8 Derivatives of Guanosine (42128)

JON R. KATZE

Department of Microbiology and Immunology, University of Tennessee Center for the Health Sciences, Memphis, Tennessee 38163

Abstract. The observed enhancement of B-lymphocyte activation by 8-bromoguanosine and 8-mercaptoguanosine is hypothesized to occur via a "binding protein" which requires a guanine nucleoside as the *syn* conformer for productive interaction. In addition, because of the 7-substituent, Q nucleoside also is hypothesized to bind as the *syn* conformer and, therefore, to be a potential B-lymphocyte activator.

My goals here are twofold: first to introduce the modified nucleoside queuosine (Q) (Fig. 1) and the association of Q deficiency with neoplasia; then to explore the notion that the observed (1–7) enhancement of B-lymphocyte activation by 8-bromoguanosine (8BrGuo) and 8-mercaptoguanosine (8MGuo) may occur by mimicry of a normal Q-dependent function. The 7-deazaguanosine derivative Q is found in the first position of the anticodon of tRNAs for the amino acids asparagine, aspartic acid, histidine, and tyrosine (8). Q is unique because (a) it is synthesized at the polynucleotide level by the irreversible exchange of queuine (Q base) with a guanine in the first position of the anticodon (9, 10) (Fig. 2); (b) Q base is not synthesized by mammals but is provided by the diet or gut flora (11, 12); and (c) animal cells can salvage Q base, enabling its reuse after tRNA degradation during the normal turnover process (13) (Fig. 2). The physiological function of Q is unknown; however, of great interest, tRNA isolated from normal differentiated tissues is almost saturated with respect to Q, while that from neoplastic cells often is significantly undermodified with respect to Q (8, 14).

Several factors dictate the Q content of tRNA, including the tissue availability of queuine, transport rate into cells, tRNA synthesis rate, insertion rate into tRNA, tRNA half-life, and queuine salvage capability (13–16). The relative contribution of each factor may vary between organisms, tissues, and tumors. For example, in early passage, normal human fibroblasts, the tumor promoter phorbol 12,13-didecanoate inhibits queuine uptake (16), leading to a decreased Q content in tRNA (15). Moreover, a significant increase in queuine salvage is observed in later passage cells, both promoter treated and untreated, partially compensating for Q starvation in the treated cells (15). Thus, deficiencies in transport or salvage may be important determinants in the Q hypomodification of neoplasia. A less common cause of Q hypomodification is a deficiency of the enzyme which inserts queuine into tRNA. This enzyme has been reported to occur at similar levels in normal and neoplastic cells (8, 14); however, my laboratory has recently discovered a human colon adenocarcinoma cell line which lacks the enzyme entirely and, therefore, lacks Q in its tRNA (J. R. Katze, unpublished). In another association between deficient queuine insertion and neoplasia, 7-methylguanine inhibits queuine insertion into tRNA *in vitro*, causes Q hypomodification of tRNA in intact cells, and induces neoplastic transformation of Chinese hamster embryo cells in culture (17).

At present we may only speculate on the physiological significance of Q in mammalian cells. Since Q is located in the wobble position of the anticodon, much effort has been expended on identifying a translational control role for Q [see Ref. (8) for review]. There is currently no compelling evidence for such a role; although, it is intriguing that Q-lacking $tRNA^{Tyr}$ from *Drosophila* is a UAG stop codon suppressor in intact *Xenopus* oocytes, but the otherwise identical Q-containing $tRNA^{Tyr}$ is not (18). The sum of the available evidence indicates a subtle physiological role for Q, and this view is supported by studies

0037-9727/85 $1.50

with Q-deficient whole organisms. The only phenotypic changes thus far observed in an *Escherichia coli* mutant which lacks Q in its tRNA are a marked reduction in viability in the stationary phase of growth (19) and an inability to induce the synthesis of nitrate reductase (20). In addition, long-term Q-deficient (raised on a Q-free diet), germfree mice appear disappointingly normal (11, 21, 22); although they do exhibit decreased reproductive efficiency (22) and depressed leukocyte and immunoglobulin G levels (21). Therefore, while Q-containing isoaccepting tRNA species may have roles in optimizing protein synthesis and even may govern the expression of natural read-through proteins, we are justified in considering subtle alternate physiological roles for Q. One such role may be in B-lymphocyte activation.

A major conformational aspect of nucleosides is the relative orientation, about the glycosyl bond, of the base to the sugar moiety. Two conformational states are preferred: as illustrated in Fig. 1, the bulky part of the base is located over the sugar in *syn* and away from the sugar in *anti*. Normal purine nucleosides in solution undergo *anti-syn* conversion with relative ease; but this is hindered by substitution at C8, such that the bulky substituents on 8BrGuo and 8MGuo "fix" these nucleosides in the *syn* conformation (23, 24). Coincidentally, substitution of guanosine at the C8 position with either a bromine or thio group specifically endows the molecule with B-cell activation properties (1–7). The activities of 8BrGMP and 8BrcGMP are much reduced compared to 8BrGuo (1); while most other C8-guanosine derivatives, as well as 8-Br-derivatives of related purine nucleosides, are inactive (M. G. Goodman, personal communication). Therefore, assuming that 8BrGuo and 8MGuo are mimicking a normal physiological effector, what might it be?

My case for the identity of the physiological "B-cell activator" rests on the hypothesis, originally alluded to by Goodman and Weigle (2), that 8BrGuo and 8MGuo activate B lymphocytes by interaction with a protein which requires a guanosine nucleoside in the *syn* conformation for productive binding. Guanosine itself may not be active because it is too rapidly catabolized or converted to

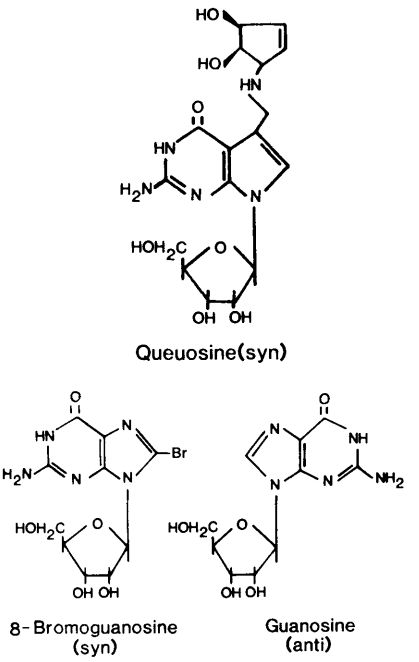

FIG. 1.

nucleotides, while in lymphocytes 8BrGuo is virtually inert to further metabolism (1). Alternatively, the "binding protein" could possess a second high affinity, but nonproductive binding site for the *anti* conformer of Guo. Finally, I propose that Q, by virtue of the additional functional groups in the methylaminocyclopentenediol-substituent at C7, also may bind as the *syn* conformer and, therefore, also may be a B-cell activator. The three-dimensional structure of Q indicates that the bulky 7-substituent is extended outward from the base and that the bond lengths and bond angles of the pyrimidine moiety of the 7-deazaguanine ring are almost equal to those of the unmodified guanine ring (25). Therefore, it is likely that the hydrogen bonding properties of the base differ little from those of guanine. Interaction of the 7-substituent of Q to the "binding protein" could cause an "induced fit" enhancement of *syn* binding affinity, inactivation of a possible Guo *anti*-binding site, or demand that Q adopt the *syn* conformation in order to also accommodate binding of the sugar and base. Of possible significance, the base-stacking property of the 7-deazaguanine moiety is significantly less than that for guanine and the mobility of

rotation about the glycosyl band is increased compared to guanosine (26). Thus, I envisage that a productive interaction of effector to the "binding protein" requires binding of (a) a ribose moiety, as well as (b) a *syn* guanine moiety and, further, that this productive interaction is enhanced by binding of (c) a moiety contained in the 7-substituent of Q. This model leads to the additional prediction that Q base might interact nonproductively with the "binding protein" and, therefore, might compete with B-cell activation. Results supporting these hypotheses have now been obtained (M. G. Goodman, personal communication), using pure Q and queuine provided by my laboratory. Briefly, Q at 10^{-4} *M* appears to be a B-cell activator, though less efficient than 8BrGuo and 8MGuo; and queuine inhibits B-cell activation. These results are preliminary and more definitive studies, which will require the isolation of larger amounts of Q, are planned. Nonetheless, considering that no other physiological nucleosides have been identified which mimic the activity of 8BrGuo and 8MGuo, these results are very encouraging.

It is troublesome, however, that normal intracellular Q concentrations probably do not exceed 10^{-9} *M*. The latter follows from the fact that most Q is derived from tRNA turnover (Fig. 2), that tRNA half-lives of 2–3 days are common (14), and that cells contain approximately 10^{-6} *M* Q in the form of tRNA [estimated from the tRNA content of tissues and the Q content of tRNA (27)]. In addition to the rate of tRNA degradation and the Q content of tRNA, the steady-state concentration of Q nucleoside would reflect its rate of formation from Q monophosphate as well as the kinase conversion to Q-5′-phosphate, the competing breakdown of Q-5′-phosphate to Q base, and efflux from the cell. The limited data available (15, 28) suggest that, in those cells with significant Q base salvage activity, there is almost no loss of intracellular Q during tRNA breakdown and resynthesis, i.e., that the process is very efficient. Accordingly, there are probably vanishingly small concentrations of free Q nucleoside in such cells. However, after phagocytosis, the Q-containing tRNA of bacteria or other cells could be broken down to the nucleoside. Thus, it is possible that Q

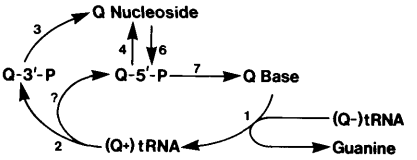

FIG. 2. Q-base salvage cycle. Step 1 is catalyzed by tRNA-guanine ribosyltransferase (EC 2.4.2.29). Steps 2–4 represent known or expected activities. Steps 6 and 7 represent activities described in Ref. (13). Step 7 is not performed by hypoxanthine-guanine phosphoribosyltransferase and Q base cannot be formed directly from Q nucleoside [Step 5 in a hypothetical Q-base salvage cycle, Ref. (13)]. (Q+)-tRNA contains Q in the 5′ position of the anticodon; (Q−)-tRNA, the precursor of (Q+)-tRNA, contains guanosine in the 5′ position of the anticodon.

nucleoside levels may reflect phagocytic activity and, if so, Q may be a logical modulator of B-cell activation. The Q base content of animal serum is 10^{-7} *M* for bovine and 10^{-8} *M* for human, with comparable levels in milk and urine [(29); J. R. Katze, unpublished], and no Q nucleoside ($<10^{-8}$ *M*) has been detected in fetal bovine serum (J. R. Katze, unpublished). At present, no data are available concerning Q or Q-5′-phosphate concentrations in lymphocytes, let alone how these might be influenced by exogenous Q or by B-cell activation.

There is also the possibility that Q-5′-phosphate rather than Q nucleoside is the physiological effector for the B-cell activator "binding protein." But, 8BrGuo must be acting as the nucleoside, because Goodman and Weigle were unable to measure significant formation of 8BrGMP from 8BrGuo (1). Moreover, since 8-bromoguanine does not mimic 8BrGuo (2), any formation of 8BrGMP would require a kinase activity, rather than purine salvage activities. The order of potency for extracellular 8-bromoguanosine derivatives is 8BrGuo > 8BrGMP > 8BrcGMP (1); however, the intracellular activity of 8BrGMP in this system is unknown. Extracellular 8BrGMP would be degraded, prior to uptake as the nucleoside, by the membrane-associated 5′-nucleotidase of B lymphocytes (1) and 8BrcGMP is not expected to be a substrate for cyclic phosphodiesterases (30, 31) and, therefore, is unlikely to efficiently generate 8BrGMP intracellularly.

Finally, I should note that Q exists in tRNA not only as the free nucleoside but also as galactosyl-Q in $tRNA^{Tyr}$ and mannosyl-Q in $tRNA^{Asp}$ (the hexose moieties are attached to the ortho hydroxyl of the cyclopentenediol-substituent) (8). No function has so far been ascribed to these Q derivatives, the hexose moieties of which are efficiently removed, prior to queuine salvage, during tRNA turnover (13).

The simple model based on a *syn* conformation imposed by bulky 8-substituents has provided not only a plausible explanation for the specificity of 8BrGuo and 8MGuo in B-lymphocyte activation but also an apparently accurate prediction that Q may be a physiological B-cell activator. However, 8-aminoguanosine is inactive in B-cell activation, while 8-oxyguanosine is active (M. G. Goodman, personal communication), even though both substituents are small enough to permit *syn-anti* conversion (23, 32, 33), indicating that other factors must also be involved. In this regard, it has been proposed that in addition to size the electronegativity of the substituent also contributes to the *syn* conformation in purines (34). The notion (23) that the conformation at the glycosyl bond affects nucleoside interactions, especially when functional groups in both the sugar and base are recognized, is amply supported. In fact, it has been generalized that enzymes which alter the glycosidic tortional angle of their nucleotide substrates are highly specific for these substrates (35). Thus, specificity is increased by the requirement for specific conformational mobility in addition to specific functional groups.

1. Goodman MG, Weigle WO. Activation of lymphocytes by brominated nucleoside and cyclic nucleotide analogues: Implications for the "second messenger" function of cyclic GMP. Proc Natl Acad Sci USA **78**:7604–7608, 1981.
2. Goodman MG, Weigle WO. Induction of immunoglobulin secretion by a simple nucleoside derivative. J Immunol **128**:2399–2404, 1982.
3. Goodman MG, Weigle WO. Activation of lymphocytes by a thiol-derivatized nucleoside: Characterization of cellular parameters and responsive subpopulations. J Immunol **130**:551–557, 1983.
4. Goodman MG, Weigle WO. Derivatized guanosine nucleosides: A new class of adjuvant for in vitro antibody responses. J Immunol **130**:2580–2585, 1983.
5. Goodman MG, Weigle WO. Manifold amplification of *in vivo* immunity in normal and immunodeficient mice by ribonucleosides derivatized at C8 of guanine. Proc Natl Acad Sci USA **80**:3452–3455, 1983.
6. Goodman MG, Weigle WO. Immunopotentiation by C8-substituted guanine ribonucleosides. Proc Soc Exp Biol Med, in press.
7. Goodman MG, Weigle WO. Mechanism of 8-mercaptoguanosine-mediated adjuvancy: Roles of clonal expansion and cellular recruitment. J Immunol **133**: 2910–2914, 1984.
8. Nishimura S. Structure, biosynthesis, and function of queuosine in tRNA. Prog Nucleic Acid Res Mol Biol **28**:49–80, 1983.
9. Katze JR, Farkas WR. A factor in serum and amniotic fluid is a substrate for the tRNA-modifying enzyme tRNA-guanine transferase. Proc Natl Acad Sci USA **76**:3271–3275, 1979.
10. Shindo-Okada N, Okada N, Ohgi T, Goto T, Nishimura S. Transfer ribonucleic acid guanine transglycosylase isolated from rat liver. Biochemistry **19**: 345–400, 1980.
11. Reyniers JP, Pleasants JR, Wostmann BS, Katze JR, Farkas WR. Administration of exogenous queuine is essential for the biosynthesis of queuosine containing transfer RNA in the mouse. J Biol Chem **256**:11,591–11,594, 1981.
12. Katze JR, Gündüz U, Smith DL, Cheng CS, McCloskey JA. Evidence that the nucleic acid base queuine is incorporated intact into tRNA by animal cells. Biochemistry **23**:1171–1176, 1984.
13. Gündüz U, Katze JR. Queuine salvage in mammalian cells: Evidence that queuine is generated from queuosine-5′-phosphate. J Biol Chem **259**:1110–1113, 1984.
14. Katze JR, Beck WT, Cheng CS, McCloskey JA. Why is tumor tRNA hypomodified with respect to Q nucleoside? Recent Results Cancer Res **84**:146–159, 1983.
15. Elliott MS, Katze JR, Trewyn RW. Relationship between a tumor promoter-induced decrease in queuine modification of transfer RNA in normal human cells and the expression of an altered cell phenotype. Cancer Res **44**:3215–3219, 1984.
16. Elliott MS, Trewyn RW, Katze JR. Inhibition of queuine uptake in cultured human fibroblasts by phorbol 12,13-didecanoate. Cancer Res **45**:1079–1085, 1985.
17. Elliott MS, Trewyn RW. Queuine hypomodification of tRNA induced by 7-methylguanine. Biochem Biophys Res Commun **104**:326–332, 1982.
18. Bienz M, Kubli E. Wild-type tRNA Tyr reads the TMV RNA stop codon, but Q base-modified tRNA Q Tyr does not. Nature (London) **294**:188–190, 1981.
19. Noguchi S, Nishimura Y, Hirota Y, Nishimura S. Isolation and characterization of an *Escherichia coli*

mutant lacking tRNA-guanine transglycosylase: Function and biosynthesis of queuosine in tRNA. J Biol Chem **257**:6544–6550, 1982.
20. Jänel G, Michelsen U, Nishimura S, Kersten H. Queuosine modification in tRNA and expression of the nitrate reductase in *Escherichia coli*. EMBO J **3**: 1603–1608, 1984.
21. Hashimoto K, Handa H, Umehara K, Sasaki S. Germfree mice reared on an "antigen-free' diet. Lab Animal Sci **28**:38–45, 1978.
22. Wostmann BS. The germfree animal in nutritional studies. Ann Rev Nutr **1**:257–279, 1981.
23. Ward DC, Reich E. Relationship between nucleoside conformation and biological activity. Annu Rep Med Chem, 272–284, 1969.
24. Tavale SS, Sobel HM. Crystal and molecular structure of 8-bromoguanosine and 8-bromoadenosine, two purine nucleosides in the *syn* conformation. J Mol Biol **48**:109–123, 1970.
25. Yokoyama S, Miyazawa T, Iitaka Y, Yamaizumi Z, Kasai H, Nishumura S. Three-dimensional structure of hyper-modified nucleoside Q located in the wobbling position of tRNA. Nature (London) **282**:107–109, 1979.
26. Seela F, Tran-Thi Q-H, Franzen D. Poly(7-deazaguanylic acid), the homopolynucleotide of the parent nucleoside of queuosine. Biochemistry **21**:4338–4343, 1982.
27. Kasai H, Kuchino Y, Nihei K, Nishimura S. Distribution of the modified nucleoside Q and its derivatives in animal and plant transfer RNA's. Nucleic Acids Res **2**:1931–1939, 1975.
28. Gündüz U, Katze JR. Salvage of the nucleic acid base queuine from queuine-containing tRNA by animal cells. Biochem Biophys Res Commun **109**: 159–167, 1982.
29. Katze JR, Basile B, McCloskey JA. Queuine, a modified base incorporated posttranscriptionally into eukaryotic transfer RNA: wide distribution in nature. Science (Washington, DC) **216**:55–56, 1982.
30. Miller JP, Boswell KH, Muneyama K, Simon LN, Robins RK, Shuman DA. Synthesis and biochemical studies of various 8-substituted derivatives of guanosine 3',5'-cyclic phosphate, inosine 3',5'-cyclic phosphate, and xanthosine 3',5'-cyclic phosphate. Biochemistry **12**:5310–5319, 1973.
31. Wells JN, Wu YJ, Baird CE, Hardman JG. Phosphodiesterases from porcine coronary arteries: Inhibition of separated forms by xanthines, papavarine, and cyclic nucleotides. Mol Pharmacol **11**:775–783,1975.
32. Miles DW, Townsend LB, Robins MJ, Robins RK, Inskeep WH, Eyring H. Circular dichroism of nucleoside derivatives. X. Influence of solvents and substituents upon Cotton effects of guanosine derivatives. J Amer Chem Soc **93**:1600–1608, 1971.
33. Jordan F, Niv H. C8-Amino purine nucleosides, a well-defined steric determinant of glycosyl conformational preferences. Biochim Biophys Acta **476**: 265–271, 1977.
34. Birnbaum GI, Shugar D. A purine nucleoside constrained in the *syn* form, crystal structure and conformation of 8-(α-hydroxyisopropyl)-adenosine. Biochim Biophys Acta **517**:500–510, 1978.
35. Mildvan AS, Kiser ET, Rosevear PR, Bramsan HN. NMR studies of the mechanism of cyclic AMP-dependent protein kinase. Fed Proc **43**:2634–2639, 1984.

Received January 28, 1985. P.S.E.B.M. 1985, Vol. 179.
Accepted March 6, 1985.