Environmental Factors and Aryl Hydrocarbon Hydroxylase Activity (CYP1A1 Phenotype) in Human Lymphocytes

Chikako Kiyohara¹ and Tomio Hirohata²

Since aryl hydrocarbon hydroxylase (AHH) is considered to be responsible for the activation of benzo(a)pyrene (BP) and other polyaromatic hydrocarbons in cigarette smoke to carcinogens, it is important to examine AHH activity in the determination of susceptibility to lung cancer. Lymphocytes from healthy male adults (239) of non-smokers and smokers were cultured in vitro and assayed for non-induced and 3-methylcholanthrene (MC)-induced AHH activity and AHH inducibility (MC-induced AHH activity/non-induced AHH activity). A day-to-day variation in AHH activity was not observed while a seasonal variation was apparent. Very wide differences in non-induced AHH and MC-induced AHH activities were observed. The association of some selected environmental factors and AHH activity was studied. Age was related to non-induced AHH activity (Spearman's rank correlation coefficients (r), r=0.185, p<0.005) and AHH inducibility (r=-0.329, p<0.001). Coffee consumption was associated with non-induced (age-adjusted r=0.138, p<0.05) and MC-induced AHH activity (age-adjusted r=0.173, p<0.01). Cigarette smoking was correlated with non-induced AHH activity (age-adjusted r=0.191, p<0.005) and AHH inducibility (age-adjusted r=-0.191, p<0.005). No significant association was observed for any other selected factors, including alcohol intake or broiled food consumption. In conclusion, AHH activity might be affected by cigarette smoking and coffee consumption, and was dependent on the age of the donor. Day-to-day and seasonal variation analyses showed that this assay method was reproducible and reliable and AHH inducibility might be a useful biomarker in cancer epidemiology. As those factors may affect the AHH activity, a careful control of those factors to AHH activity is necessary in epidemiological studies on the association between AHH inducibility in human lymphocytes and lung cancer. 

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AHH (aryl hydrocarbon hydroxylase) is a membrane-bound monooxygenase system located in most tissues of the body. In mice, AHH inducibility (AHH activity in 3-methylcholanthrene (MC)-treated mice/AHH activity in olive oil (vehicle)-treated mice) is under the control of the Ah locus and certain inbred strains of mice are susceptible to AHH induction by MC treatment (Ah responsive strains), while other strains are not (Ah non-responsive strains). Although a strong correlation was observed between AHH inducibility and tumor incidence in mice, the effect of AHH induction on tumorigenesis depends on the effects of the inducer on both metabolic activation and detoxification, the animal model and the carcinogen administered. Thus, the effect of AHH induction on tumorigenesis is complex. Since AHH is considered to be responsible in humans for the activation of benzo(a)pyrene (BP) and other polyaromatic hydrocarbons (PAHs) in cigarette smoke to carcinogens, it may also be important in the causation of lung cancer.

Much effort has been made to determine the relationship between AHH activity or inducibility and susceptibility to lung cancer or other forms of cancer in human populations as well, but the results were not very consistent. The main reason for this inconsistency may have been difficulty in the measurement of AHH activity in lymphocytes. AHH activity might be affected by the conditions of cell culture. Fetal calf serum may contain inducers of AHH activity that vary with the particular

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lot of serum, thereby rendering the apparent induction ratio an imprecise indicator of genetic susceptibility to induction by MC. In addition, it might be also affected by many environmental factors. Little is known about the effects of environmental influences on AHH activity. Lastly, the determination of the AHH activity is very laborious and cumbersome because the procedure requires more than 20 ml of blood, the isolation and culturing of lymphocytes for 4 days, and a spectrophotofluorometric assay involving hazardous chemical. To the best of our knowledge, this is the first report on the environmental factors on AHH activity in human lymphocytes. If the influential environmental factors on AHH activity could be elucidated and then AHH activity and/or AHH inducibility could be assessed precisely, it would be helpful in predicting individual risk of cancer and toxicity.

In this study, to clarify the role of AHH activity in carcinogenesis, we investigated the effect of environmental factors on non-induced, MC-induced AHH activity and AHH inducibility in cultured lymphocytes from healthy adult volunteers.

**MATERIALS AND METHODS**

**Chemicals**

3-Hydroxybenzo (a) pyrene (3-OHBP) was kindly provided by Dr. N. Kinoshita, School of Health Sciences, Kyushu University, Fukuoka. BP was purchased from Sigma Chemical Co. (St. Louis, MO) and recrystallized from methanol solution. RPMI-1640 medium was obtained from Nippon Biochemical Laboratory (Kyoto, Japan). All other chemicals and reagents were of the highest quality commercially available.

**Subjects and sample collection**

Two hundreds and twenty-nine Japanese healthy male volunteers (employees of Fukuoka prefectural government), aged 20 to 68 (mean 40.43 years), participated in this study from April 1993 to March 1995. The subjects gave written informed consent and completed a short questionnaire on their current lifestyle characteristics. The examined factors were suspected to influence on AHH activity. They were vegetable and fruit intake (times/week), coffee consumption (cups/day), milk consumption (times/week), broiled meat (times/week), broiled fish (times/week), smoking habit (cigarettes/day) and alcohol drinking (times/week), heating system and ventilation, and residential area (adjacent to a road where there is much traffic or not). They consisted of both smokers and nonsmokers. None of the subjects in this study were under medication. Heparin (40 IU/ml) was used as an anticoagulant and the blood was generally processed within 2 to 3 hr after collection (time of blood collection: 12:00-13:00 p.m.). In addition, 10 males, who were included in the above population, were used for the study on the day-to-day and seasonal variations of AHH activity.

**Separation of lymphocytes**

Fifteen ml of Ficoll-Conray solution (specific gravity, 1.077) was previously placed in a lymphocyte-separation tube LeucoSep (Greiner GmbH, Frickenhausen, Germany). Then 20 ml of blood sample was poured into the tube and spun at 1,000 x g for 10 min at room temperature. After centrifugation, the plasma layer was aspirated with Pasteur pipette and then a lymphocyte-rich fraction was collected. The lymphocyte-rich fraction was mixed with 10 ml of 0.01M phosphate-buffered saline (PBS, pH 6.8) and then centrifuged at 250 x g for 10 min at room temperature. The step was repeated twice. The purified, washed and counted (1 X 10^6 cells/ml) lymphocytes were resuspended in complete RPMI-1640 medium (penicillin 100 μg/ml, streptomycin 50 μg/ml, and phytohemagglutinin and pokeweed mitogen; both diluted 1:100 with distilled PBS, 20% heat-inactivated fetal bovine serum). Ten ml of cell suspension in a flask was incubated at 37°C for 96 hr in an atmosphere of fully humidified air with 5% CO₂.

**AHH assay**

At 48 hr, 5 μl of MC in acetone, to give a final concentration of 2.5 μM, was added to the cell culture (to obtain the MC-induced AHH activity). In a control culture (to measure the non-induced AHH activity), the solvent (acetone) alone was added (5 μl/10 ml culture medium). Incubation was continued for an additional 48 h period. The cells from the culture flasks with a viability of 90% or more were harvested, washed twice with 0.05 M Tris-HCl buffer supplemented with 0.2 M sucrose and 3 mM MgCl₂. AHH activity was determined by a modification of the method of Kiyohara et al.

By using purified lymphocytes and Nikken RPMI-1640 medium, harvesting the cells at the time of minimum cell death and maximum AHH activity, employing quinine sulfate solution as working standard and assaying AHH at its pH optimum of 8.5, it is possible to enhance the sensitivity and the reproducibility of the assay.

**Statistical analysis**

Statistical analysis was performed with the PC/SAS statistical package (SAS Institute, Cary/NC). Spearman's rank correlation coefficients (r) were used to determine the univariate relation between the AHH activity and the selected dietary factors. Potential confounding effects of age and other factors were accommodated by calculating Spearman's partial rank correlation coefficients and by performing multiple regression analyses. Since Non-induced and MC-induced AHH activity were skewed to the right side, log-transformed values of these were used in calculating the means and 95% confidence intervals (CI) and in regression analyses. Adjusted means were derived from the General Linear Models (GLM) procedure. All the P values are two-sided and the P values less than 0.05.
Table 1. Inducibility of Aryl Hydrocarbon Hydroxylase (AHH) in Lymphocytes from 239 Human Subjects.

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>40.43</td>
<td>20-68</td>
</tr>
<tr>
<td>Non-induce AHH activity&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.043</td>
<td>0.005-0.308</td>
</tr>
<tr>
<td>MC-induced AHH activity&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.174</td>
<td>0.012-1.244</td>
</tr>
<tr>
<td>AHH inducibility (MC/Non)&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>4.03</td>
<td>1.23-20.80</td>
</tr>
</tbody>
</table>

<sup>a</sup> Geometric values are shown due to the right-skewed distribution of the corresponding variables.
<sup>b</sup> Value relative to the AHH activity (3-hydroxy BP formed, pmol/min/10<sup>6</sup> cells) of the acetone-treated cells.

were considered statistically significant.

**RESULTS**

**Day-to-day and seasonal variation in AHH activity**

A day-to-day variation was observed when AHH activity was measured in lymphocytes of 10 male subjects. The improvements to the technique which has been shown high reproducibility of AHH inducibility (MC-induced AHH activity/non-induced AHH activity), as well as non-induced and MC-induced AHH activities, over a period of 3 consecutive days (data not shown). Particularly, the level of reproducibility of AHH activity was very high. Next, AHH activity was measured every 3 months (every seasons). AHH activity did not remain constant throughout the year. A significant seasonal variation was observed with non-induced and MC-induced AHH activities; non-induced and MC-induced AHH activities showed the highest value in summer and the lowest in winter (data not shown). Consequently, AHH inducibility was more stable. Furthermore, this tendency was also observed when the subjects (n=239) were divided into four seasons according to the date of blood collection.

**Interindividual differences**

Table 1 shows the non-induced, MC-induced AHH activities, and AHH inducibility in peripheral lymphocytes from 239 male subjects, who ranged in age from 20 to 68 years. A very

Table 2. Rank Correlation coefficients<sup>a</sup> of AHH activity with selected factors.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Specific AHH activity</th>
<th>AHH inducibility</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Crude</td>
</tr>
<tr>
<td>Age</td>
<td>0.185***</td>
<td>-</td>
</tr>
<tr>
<td>Cigarette</td>
<td>0.191***</td>
<td>0.205***</td>
</tr>
<tr>
<td>Coffee</td>
<td>0.079</td>
<td>0.138*</td>
</tr>
<tr>
<td>Alcohol</td>
<td>-0.016</td>
<td>-0.043</td>
</tr>
<tr>
<td>Broiled meat</td>
<td>-0.005</td>
<td>0.017</td>
</tr>
<tr>
<td>Broiled fish</td>
<td>0.036</td>
<td>0.019</td>
</tr>
</tbody>
</table>

<sup>a</sup> Spearman's rank correlation coefficients.
<sup>b</sup> Adjusted for age.
<sup>*p<0.05,**p<0.01,***p<0.005,****p<0.001</sup>

Table 3. AHH activity and AHH inducibility in relation to age class.

<table>
<thead>
<tr>
<th>Age class</th>
<th>N</th>
<th>Specific AHH activity&lt;sup&gt;a&lt;/sup&gt;</th>
<th>AHH inducibility</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Non-induced</td>
<td>MC-induced</td>
</tr>
<tr>
<td>20-29</td>
<td>39</td>
<td>0.034(0.038-0.043)</td>
<td>0.165(0.130-0.209)</td>
</tr>
<tr>
<td>30-39</td>
<td>92</td>
<td>0.040(0.035-0.045)</td>
<td>0.189(0.162-0.220)</td>
</tr>
<tr>
<td>40-49</td>
<td>52</td>
<td>0.048(0.040-0.057)*</td>
<td>0.185(0.151-0.226)</td>
</tr>
<tr>
<td>50+</td>
<td>56</td>
<td>0.052(0.043-0.062)**,#</td>
<td>0.148(0.122-0.180)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Expressed in terms of 3-hydroxy BP (pmol/min/10<sup>6</sup> cells) formed.
<sup>Adjusted for season.</sup>
As compared with 20-29 age class (*p<0.05,**p<0.005,***p<0.0001).
As compared with 30-39 age class (##p<0.0001).
As compared with 40-49 age class (#p<0.01).
wide range in non-induced AHH activity (0.005-0.308 pmol/min/10^6 cells; geometric mean of 0.043 pmol/min/10^6 cells) was observed. The highest non-induced AHH activity was approximately 62 times the lowest activity. Very wide ranges were also observed in MC-induced AHH activity and AHH inducibility; namely treatment with MC increased the enzyme activity from 1.23- to 20.8-fold (geometric mean, 4.03-fold), resulting in MC-induced AHH activities of 0.012-1.244 pmol/min/10^6 cells (geometric mean, 0.174 pmol/min/10^6 cells).

**Rank correlation coefficients of AHH activity and some selected factors**

Table 2 presents crude and age-adjusted correlation of selected dietary variables with AHH activity. Age was positively associated with non-induced AHH activity ($r=0.185$, $p<0.005$) and negatively with AHH inducibility ($r=-0.329$, $p<0.001$). Cigarette smoking was positively correlated with non-induced AHH activity (age-adjusted $r=0.205$, $p<0.005$) and negatively with AHH inducibility (age-adjusted $r=-0.188$, $p<0.005$). Coffee consumption was positively related to non-induced (age-adjusted $r=0.138$, $p<0.05$) and MC-induced AHH activity (age-adjusted $r=0.173$, $p<0.01$).

**Aging and AHH activity**

Table 3 demonstrates the extent of lymphocyte season-adjusted AHH activity and AHH inducibility as a function of age class, which was divided into 4 classes, namely 20-29, 30-39, 40-49 and 50+ years. The MC-induced AHH activity was constant for 20-65 years. The constitutive activity significantly increased with advancing age while MC-induced activity showed the reverse change. Accordingly, AHH inducibility was significantly decreased with advancing age. The AHH inducibility of the oldest age class (2.87-fold) is approximately one-half of that of the youngest age class (4.89). AHH inducibility was not significantly differed between the youngest age class and the following age class. Age and season were adjusted for unless otherwise specified.

**Cigarette Smoking and AHH activity**

The number of cigarette smoked per day was not dependent on the age. A dose-response relationship between adjusted AHH activity and number of cigarettes smoked per day was not strong. We divided into 3 groups according to smoking dose, namely non-smoker (including ex-smoker), light-smoker (1-19 cigarettes/day) and heavy-smoker ($\geq$20 cigarettes/day) (Table 4). Non-induced AHH activity in current smokers, especially in light-smokers, was significant higher than that in

**Table 4. Adjusted AHH activity and AHH inducibility in relation to smoking dose.**

<table>
<thead>
<tr>
<th>cigs./day</th>
<th>N</th>
<th>Specific AHH activity</th>
<th>AHH inducibility*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Non-induced</td>
<td>MC-induced</td>
</tr>
<tr>
<td>0</td>
<td>164</td>
<td>0.039(0.035-0.043)</td>
<td>0.171(0.153-0.191)</td>
</tr>
<tr>
<td>1-19</td>
<td>45</td>
<td>0.059(0.078-0.071)*</td>
<td>0.179(0.144-0.223)</td>
</tr>
<tr>
<td>20+</td>
<td>30</td>
<td>0.049(0.038-0.062)</td>
<td>0.181(0.138-0.238)</td>
</tr>
</tbody>
</table>

a Values relative to the AHH activity (3-hydoxy BP formed, pmol/min/10^6 cells) of the acetone treated cells.
Adjusted for age and season.
As compared with non-smoker (*p<0.05).

**Table 5. Adjusted AHH activity and AHH inducibility in relation to coffee intake.**

<table>
<thead>
<tr>
<th>Coffee intake</th>
<th>N</th>
<th>Specific AHH activity</th>
<th>AHH inducibility</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Non-induced</td>
<td>MC-induced</td>
</tr>
<tr>
<td>0</td>
<td>79</td>
<td>0.037(0.032-0.044)</td>
<td>0.149(0.126-0.176)</td>
</tr>
<tr>
<td>1</td>
<td>74</td>
<td>0.043(0.037-0.050)</td>
<td>0.175(0.148-0.207)</td>
</tr>
<tr>
<td>2+</td>
<td>30</td>
<td>0.050(0.040-0.064)*</td>
<td>0.208(0.161-0.268)*</td>
</tr>
<tr>
<td>3+</td>
<td>30</td>
<td>0.044(0.035-0.057)</td>
<td>0.174(0.133-0.227)</td>
</tr>
<tr>
<td>4+</td>
<td>23</td>
<td>0.055(0.042-0.073)*</td>
<td>0.229(0.162-0.296)*</td>
</tr>
</tbody>
</table>

a Values relative to the AHH activity (3-hydoxy BP formed, pmol/min/10^6 cells) of the acetone treated cells.
Adjusted for age and season.
As compared with non consumer (*p<0.05).
non-smokers (p<0.05). MC-induced AHH activity was not different among the groups. Therefore, AHH inducibility in light-smoker was significantly lower than that in non-smokers.

**Coffee consumption and AHH activity**

In analogy with cigarette consumption, there was not an excellent dose-response relationship between adjusted AHH activity and coffee consumption (Table 5). Two or, 4 or above per day consumer produced higher non-induced and MC-induced AHH activity than none consumer. Unexpectedly AHH inducibility was constant regardless of coffee consumption.

**The results of multiple regression analysis**

In order to examine the independent relation of dietary factors to AHH activity, multiple regression analysis was performed with age, smoking dose, alcohol intake, broiled meat, broiled fish and season included always in the models as independent variables. Adjusted average relative change (%) per unit was shown in Table 6. Results of multiple regression analyses were almost the same as those noted in the correlation analysis. The positive association between age and non-induced AHH activity (1.3 %/year) and the negative association between age and AHH inducibility (1.6 %/year) remained strong. Coffee intake also remained a significant correlate of MC-induced AHH activity (8.8%/cup) and AHH inducibility (5.7 %/cup). Cigarettes smoking was also related to AHH inducibility (-1.1 %/one cigarette).

**DISCUSSION**

Lung cancer mortality has been increasing rapidly in recent years in Japan and has exceeded stomach cancer mortality in male Japanese. Although chronic inhalation of cigarette smoke is a major risk factor to the development of lung cancer, it seems important to examine genetic susceptibility to the disease as well. Molecular epidemiology of cancer involves the use of biomarkers of exposure and response in studies of exogenous or endogenous agents and/or host factors that play a role in human cancer etiology. This approach has the potential for identification of susceptible individuals. The individual differences in genetic susceptibility to lung cancer may be accounted for by the activity of the drug-metabolizing enzyme AHH. The relationship between high AHH inducibility and high lung cancer incidence has been suggested.

In this study, AHH activity and AHH inducibility were determined in lymphocyte in 164 male current-smokers and 75 never-smokers. A day-to-day and a seasonal variations were checked. AHH activity showed that the reproducibility of the measurements is good and the applied assay method can therefore be used in large-scale prospective population investigations. And we also confirmed that our AHH activity assay method is reliable. Especially, the level of reproducibility of AHH inducibility was very high so that it would be useful for quantitative genetic purposes.

In previous study, we examined the relationship between AHH activity and the frequency of a MspI mutation in the 3'-flanking region of cytochrome P450 (CYP) 1A1 (MspI polymorphism) and another mutation in exon 7 (Ile-Val polymorphism) in 84 healthy male subjects in Fukuoka, Japan. It suggests that a homozygote rare allele (genotype C) of MspI polymorphism and Val/Val genotype of Ile-Val polymorphism, which are more frequent in smoking-related lung cancer, are closely related with high AHH inducibility and high non-induced AHH activity, respectively. Thus, these findings support the positive relationship between AHH inducibility and lung cancer. From this result and the result of the present study, it would be possible to identify smokers who have genetically high susceptibility to lung cancer (genotype C or Val/Val) and to prevent lung cancer by early detection.

The effects of smoking on AHH activity in various human tissues were discussed. In the placenta, induction of AHH activity by smoking has been demonstrated unequivocally while...
the enzyme activity in the lung samples is variable\(^{21,22,25}\). Cigarette smoking might also induce AHH activity in the liver\(^{21,22,26}\) and peripheral lymphocytes\(^{6,22,27}\). Our previous study suggested that the effect of cigarette smoking on AHH activity was little\(^{14}\). The effect is retained even after culturing peripheral lymphocytes but this phenomenon has not been clarified. In multiple regression analysis, the inducing effect of cigarette smoking on non-induced AHH activity was observed while that on both MC-induced AHH activity and AHH inducibility was not (Table 6). It has been pointed out that the relationship between cigarette dose and AHH activity/AHH inducibility might not be so strong and AHH inducibility may be determined by genetic factor rather than environmental exposure\(^{10}\).

Except for cigarette smoking, little is known about the effects of environmental factors on AHH activity in not only lymphocytes but also other tissues. The only one study, in which biopsies were obtained from patients who were suspected liver disease, reported that excess of alcohol drinking (more than 250g ethanol per week) was associated with decreased hepatic AHH activity and a positive correlation between the number of meat meals eaten per week\(^{20}\). In the present study, AHH activity of lymphocytes did not remained to be the correlate of broiled foods and positively, but not significantly, associated with the frequency of alcohol intake (Table 6). The effect of environmental factors on AHH activity may differ among the type of tissues. The effect of alcohol drinking on AHH activity needs to be investigated further in relation to the type of alcoholic beverage and the content of ethanol.

Both non-induced and MC-induced AHH activities were higher in coffee consumer than those in none consumer (Table 5). The coffee-specific diterpenes cafestol and kahweol, which comprise up to about 10-15% of the lipid fraction of roasted coffee beans, have recently been shown to possess several important biological activities. For example they modulate serum cholesterol levels\(^{3,29}\) and they have been reported to have anticarcinogenic properties\(^{19}\). The latter activity may be related to their ability to induce glutathione S-transferase (GST)\(^{18,38}\). However, little is known concerning their effects on other xenobiotic metabolizing and detoxifying systems. PAHs have been shown to induce coordinately, via a functional Ah receptor, at least six genes that belong to the [Ah] gene battery: two phase I genes, CYP1A1 and CYP1A2 and four phase II genes, NADPH:quinoideoxoreductase, aldehyde dehydrogenase, UDP-glucuronosyltransferase and GST\(^{20}\). Moreover, it has been reported that coffee intake (more than 4 cups of coffee) increases hepatic AHH activity and the major environmental influence on AHH activity is caffeine intake\(^{20}\). In the present study, a dose-response relationship between AHH activity and the number of cups of coffee consumed per day was not so strong. This result may be explained by rather small number of subjects tested. Additional research is needed to examine the dose-response relationship between them.

Our study showed the seasonal variation of AHH activity in 4-days cultured human lymphocytes (data not shown). Large seasonal variations in AHH activity\(^{28}\), as well as several immune functions\(^{39}\), have been reported in human beings. Seasonal variations are commonly explained by environmental changes. Namely, there is a seasonal variation which might be mainly influenced by food intakes. Nonetheless, endogenous circannual rhythm in numerous variables have been demonstrated in animals kept in a standardized environment for several years. With regard to immune functions, the lymphocyte blastogenic response of mice exhibited a reproducible circannual rhythm for 2 consecutive years despite the fact that these animals had been kept in the same photoperiodic and nutritive environment\(^{30}\).

In conclusion, age was related to non-induced AHH activity and AHH inducibility. Coffee consumption was associated with non-induced and MC-induced AHH activity. Cigarette smoking was correlated with both non-induced AHH activity and AHH inducibility. No significant association was observed for any other selected factors, including alcohol intake or broiled/grilled food consumption. Therefore, AHH activity was affected by cigarette smoking and coffee consumption, and was dependent on the age of the donor. The present study suggests that AHH inducibility may be a useful biomarker in cancer epidemiology. The public health implications deserve further investigations.

ACKNOWLEDGMENTS

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REFERENCES